THE EFFECTS OF METHOD OF PROPAGATION, AGE OF TRANSPLANTING, AND PRE-TRANSPLANTING TREATMENTS ON GROWTH AND N_2-FIXATION OF LEUCAENA LEUCOCEPHALA (LAM.) DE WIT

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ABSTRACT

The effects of method of propagation, age of transplanting, and several pre-transplanting treatments on early growth and N_2-fixation of greenhouse-grown Leucaena leucocephala (Lam.) de Wit were examined in this study. Transplanting of leucaena from dibble tubes did not compromise growth and N_2-fixation relative to plants directly-seeded into a clay soil in pots. In fact, the efficiency with which N_2 was fixed was much higher in transplanted leucaena. An age of 62 days was found to be most practical for transplanting leucaena raised in dibble tubes. Strain TAL582SR, the Rhizobium used to inoculate leucaena, formed very few root nodules in the soil (most nodules were formed by indigenous rhizobia). Strain TAL582SR may have survived poorly in the soil or could not compete with indigenous rhizobia. Among several pre-transplanting treatments utilized, heavy inoculation with a mycorrhizal fungus, Glomus mosseae, proved to be the best treatment for improving growth of leucaena transplanted at 4 weeks of age.

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I. INTRODUCTION

Leucaena is a genus which consists of about 10 species, the most extensively studied being Leucaena leucocephala (Lam.) de Wit. "Leucaena" is also one of the names found in common usage for this plant. It is a tropical, woody legume with a high degree of drought resistance (Vietmeyer and Cottom, 1979). One of leucaena's greatest assets, shared by other leguminous plants, is its ability to enter into a symbiotic association with a N_2-fixing bacterium called Rhizobium. The bacteria infect the roots of the leucaena plant, forming nodules which are most often confined to the upper levels of the soil (Vietmeyer and Cottom, 1979). Leucaena is quickly attaining a reputation as one of the most versatile and beneficial crops in the tropics. Research over the last couple of decades has shown that leucaena can be exploited for soil improvement, intercropping, forage, wood, paper pulp, fuel, human food, and reforestation (Brewbaker and Hutton, 1979). In the area of soil improvement, the leucaena-Rhizobium association is quite important. This symbiosis is capable of fixing greater than 500 kg of N_2 $ha^{-1}yr^{-1}$ under optimal conditions (Date, 1977a; Vietmeyer and Cottom, 1979). When leucaena die or shed their leaves, the nitrogenous compounds are returned to the earth, thereby enriching the soil. The planting of leucaena is invaluable in reducing the need for supplementary N fertilizers which are made increasingly expensive by spiraling energy prices.

Leucaena can be propagated both by direct-seeding and transplanting. In Hawaii and many other tropical areas, leucaena is commonly transplanted for forest establishment (Brewbaker and Hutton, 1979). Although leucaena can accumulate biomass with an unusual rapidity, it is, like

legumes, initially a slow-growing plant (Vietmeyer and Cottom, 1979). Transplanting of nursery-grown leucaena can give the seedlings a growth advantage over direct-seeded plants whose field establishment could be jeopardized by fast-growing weed species (Brewbaker and Hutton, 1979). While the transplanting method of plant establishment has its advantage, some species are adversely stressed by the transfer to a new environment (Adriance and Brison, 1939). As of this writing, the author is unaware of any other published studies which have compared direct-seeding and transplanting methods of propagation with regard to the establishment of leucaena and maintenance of its N_2-fixing symbiont. If leucaena is propagated by transplanting, it will be valuable to know if the age of transplanting can affect growth and if pre-transplanting treatments can improve seedling performance and help maintain the Rhizobium symbiosis after transplanting.

The objectives of this study were:

(1) To compare transplanting and direct-seeding methods of propagating leucaena and to define the importance of seedling age at transplanting on the early post-transplanting performance of leucaena and its symbiosis with Rhizobium.

(2) To determine the influence of a variety of pre-transplanting treatments on the early post-transplanting establishment and N_2-fixation potential of leucaena seedlings.

II. LITERATURE REVIEW

A. Leucaena

Leucaena leucocephala (Lam.) de Wit is a native to Mexico and Central America but has been naturalized in many of the tropical regions of the world between 30°N and 30°S latitudes (Brewbaker and Hutton, 1979). Leucaena is a member of the family Leguminosae and the sub-family Mimosoidae. This self-pollinating perennial is noted for its arboreal habit, characteristic leguminous seed pods, round-headed regular flowers, and compound leaves with very fine leaflets (Brewbaker and Hutton, 1979). The breeding cycle is 10 to 15 months from seed to seed (Brewbaker and Hutton, 1979). Cross-fertilization between Leucaena species has been reported and the extent of this phenomenon varies with the species (Vietmeyer and Cottom, 1979).

Leucaena varies widely in size and form with over one hundred varieties known (Vietmeyer and Cottom, 1979). The present taxonomic classification allows leucaena to be separated into three types: "the Hawaiian", "the Salvador", and "the Peru" type. The "Salvador" type is a tall (to 20 m), high yielding plant often producing double the photosynthate of the "Hawaiian" type (Vietmeyer and Cottom, 1979). The K8 variety of the "Salvador" type has a promising yield potential and is being planted in numerous field trials in Hawaii. Whitney (1977) noted that the K8 variety can produce in excess of 23,000 kg $ha^{-1}yr^{-1}$ and 900 kg N $ha^{-1}yr^{-1}$. Even though it is limited to the tropics and subtropics, leucaena tolerates a fairly wide range of environments. According to Vietmeyer and Cottom (1979), this legume withstands large differences in rainfall, sunlight, salinity, as well as periodic inundation, fire,

windstorms, slight frost, and drought. The optimum range of temperature for leucaena growth is between 25°C and 30°C with little growth occurring below 10°C (Brewbaker and Hutton, 1979). A deeply penetrating taproot aids leucaena in resisting drought-induced desiccation. Leucaena performs best in a neutral or alkaline soil, growing poorly in many of the acidic soils of the tropics (Brewbaker and Hutton, 1979).

B. The Leucaena-*Rhizobium* Symbiosis

Leucaena is benefited by the presence of N_2-fixing *Rhizobium* which form nodules on small root laterals near the soil surface. These rhizobia are generally fast-growing, acid-producing strains (Date, 1977b). The nodules are often multilobed and 2.5 to 15 mm in diameter (Vietmeyer and Cottom, 1979). Effective nodules are reddish-pink inside due to the presence of leghaemoglobin which is produced by the host plant (Alexander, 1977a). Conversely, *Rhizobium* possesses all the genes for nitrogenase synthesis and activity (Dilworth and McComb, 1977). Two methods often used to estimate nitrogenase activity are the acetylene reduction method (Vincent, 1970; Asimi et al., 1980) and the use of ^{15}N-tagged N_2 (Alexander, 1977a; Talbott et al., 1982).

The leucaena-*Rhizobium* symbiosis is not extremely specific since Trinick (1968) reported that leucaena *Rhizobium* can effectively nodulate *Vigna* spp. Rhizobia capable of nodulating leucaena have been isolated from other tropical legumes such as *Mimosa invisa*, *M. pudica*, *Acacia farnesiana*, and *Sesbania grandiflora* (Trinick, 1980). Date (1977b) placed *L. leucocephala* into Group S, a category of tropical pasture legumes that nodulate effectively only with specific strains. However, he concedes

that L. leucocephala may more correctly belong to Group PI. In Group PI, the legumes may nodulate with several strains of Rhizobium but only a few of the symbioses will fix adequate amounts of N_2 to meet host requirements.

If effective rhizobia are absent from the soil, many tropical legumes are unable to compete with other plant species (Jones, 1977; Keya, 1977). However, the proper rhizobia are present in many tropical soils and effective nodulation of leucaena often occurs naturally (Brewbaker and Hutton, 1979). Gaur and Lowther (1982) reported that effective strains of rhizobia differ in their ability to nodulate the host and to persist in competition with naturally-occurring rhizobia. For example, L. leucocephala cv. Peru planted in Australia was shown to nodulate in the absence of inoculation. When the yields of the uninoculated cultivar were compared to the cultivar inoculated with an effective strain, NGR8, they were found to be identical. Serological tests showed that the strain present in the nodules of the inoculated plants was not the one used originally for inoculation (Jones, 1977). Another strain, CB81 (TAL582), has been the preferred strain for the inoculation of leucaena in Australia (Jones, 1977; Brewbaker and Hutton, 1979). Low recovery has been reported for serologically-marked Rhizobium japonicum inoculants in soils containing naturalized populations of rhizobia (Johnson et al., 1965; Ham et al., 1971). In addition to serological agglutination (Vincent, 1970), the persistance of introduced strains of Rhizobium in the soil and as a symbiont can be traced by developing an antibiotic-resistant mutant (Alexander, 1977b; Amarger, 1981a) and fluorescent antibody labelling (Schmidt et al., 1968; Trinick, 1969; Schmidt, 1973).

If legumes are not grown in a soil over a period of a few years, the level of soil-borne rhizobia can drop exponentially (Nutman and Hearne, 1980). Holland (1970) suggested that where a specific strain is desired for nodulation and competition with soil rhizobia is likely, commercial inoculants should be applied at rates exceeding those generally recommended for effective nodulation. For alfalfa, Obaton (1977) and Gibson et al. (1976) recommended 10^7 to 10^9 rhizobia per seed where indigenous bacteria are present in great numbers. Even with an initially large number of rhizobia per seed at planting, percentage recovery of the preferred strain can vary greatly. In the field, Kuykendahl and Weber (1978) found that only 5 to 10% of the soybean nodules examined contained the bacterial strain with which the seeds were inoculated. Conversely, a field experiment with crimson clover showed a high frequency (greater than 85% of the nodules sampled) of recovery for antibiotically-labelled strains of Rhizobium trifolii (Materon and Hagedorn, 1982).

No estimates of the average lifespan of a leucaena root nodule have been reported. The lifespan of the nodule is likely to vary with the health of the legume and a myriad of environmental factors. It has been shown that greenhouse-grown soybeans have a nodule longevity of 6 to 7 weeks (Friere, 1977).

C. Transplanting Seedlings

Leucaena is commonly transplanted in the tropics. Establishment of leucaena by direct-seeding can be slow and the seedling is often subject to the effects of competition from weeds (Brewbaker and Hutton, 1979). A popular container used for the pre-transplanting growth of leucaena is

the dibble tube (Walters, 1980). Dibble tubes vary in size and accomodate only one seedling. Longitudinal inner ridges in the tubes prevent root spiraling by guiding the roots of the seedlings in a downward direction. Pruning of the taproot occurs naturally at the open bottom of the tubes. In a typical procedure, leucaena seeds are inoculated with Rhizobium, placed in dibble tubes containing a mixture of peat, vermiculite, and soil, and grown until 2 or 3 months of age with the plants reaching a height of 20 to 25 cm (Brewbaker and Hutton, 1979; Walters, 1980). If the root growth is thick enough, seedling and growth medium are easily removed intact from the dibble tube and placed directly into a depression in the soil created by a dibble stick. A two-phase inoculation procedure in which leguminous plants are inoculated at both the time of planting and transplanting can sometimes be useful in maximizing the legume-Rhizobium symbiosis (Asimi et al., 1980; J. Halliday, personal communication). Poor nodulation resulting from inoculation failure in forage legumes sometimes can be corrected with properly timed supplemental soil inoculation (Rogers et al., 1982). Both procedures may be applicable to leucaena.

The containers in which the seedlings are grown prior to transplanting can influence development through the nature of their physical construction and soil-water relationships (Knavel, 1964). Also, interception of the roots by the walls of a pot may produce unrealistically high nutrient requirements (Fox and Kang, 1977). Knavel (1964) reported in a study involving tomato seedlings that the growth and development of transplants were significantly influenced by pot size and plant spacing. His results suggested that a larger pot size and increased spacing in the nursery allow faster post-transplanting growth due to increased branching

of the root system. Kratky et al. (1982) noted that larger pre-transplanting containers produced larger Chinese cabbage seedlings for transplanting.

Transplanting has been recognized to be a major shock to a young seedling's physiology. Stone (1967) has expressed the physiological state of a plant in terms of the growth rate (cell multiplication and expansion), enzymatic rates with endogenous and exogenous substrates, the concentration of specific chemicals in selected plant tissues, and the CO_2 exchange rates in the light and dark. Any one or a combination of these biological processes can be affected by transplanting.

The shock of transplanting can sometime check the growth of a seedling. The extent and duration of the inhibition varies with how drastically the new growth medium differs from the pre-transplanting medium, how much physical damage the plant sustains during the actual transplanting process, and the plant being studied (Adriance and Brison, 1939). The general symptoms of transplanting shock include wilting, decreased photosynthate production, and often a delay in maturity (Adriance and Brison, 1939; Gayed, 1971; Wang and Kratky, 1976; Wang et al., 1979). In leucaena and other leguminous plants, this category might be enlarged to include such possible symptoms as the loss of nodules or nodule effectiveness, leading to a lower overall level of N_2-fixation and plant vigor. Alexander (1977a) and Graham and Halliday (1977) noted that a considerable carbohydrate demand is made on the host legume for maintenance of the root nodules. If the legume is severely stressed at transplanting, it may not be able to deliver an adequate amount of carbohydrates to the root nodules to support N_2-fixation activities.

One of the most obvious stresses connected with transplanting is direct damage to the roots. Damage is often done through the pruning of long roots (a common practice in some species of saplings grown in nurseries) and destruction of the fine absorptive root hairs (Hartmann and Kester, 1961; Schoenweiss, 1976). The ability of a plant to recover after transplanting depends on plant reserves, the proportion of the root system retained after transplanting, and the effectiveness of the retained roots in absorbing water during the first few days thereafter (Stone, 1967). The volume of roots and root growth required to keep plants supplied with water after transplanting varies with the availability of soil moisture, the evaporative stress that the plant is subjected to, the evaporative surface exposed by the plant, and the moisture requirements of the plant being considered (Stone and Jenkinson, 1970; Walters, 1970). Goor and Barney (1968) stated that the transplanting process induces better development of the root system in several species by increasing the number of fine, absorbing rootlets which develop most profusely near the severed ends of the roots.

According to Lloyd (1914), plants with well-developed fibrous roots and a compact root system are less likely to suffer from transplanting than those in which the root system consists principally of a long, central taproot. The amount of taproot growth inhibition due to transplanting appears to vary with the species of taproot-forming plants. Upshall (1939) observed that transplanting young peach seedlings discouraged taproot development only slightly. On the other hand, Gandhi (1971) reported that improper handling of papaya seedlings during transplanting distorted the root. Direct-seeded poplars have been shown to grow twice as fast as transplanted ones, and to be richer in nutrients (Zabielski,

1969). As mentioned earlier, dibble tubes are the usual mode of raising leucaena for transplanting in Hawaii. A possible point of damage, if any, is in the air-pruning of the taproot at the open bottom of the tube. It is not known to what extent the taproot regenerates in the field. The lack of a rapidly growing taproot would be detrimental to leucaena growth in arid regions where a long taproot is a prerequisite for survival in the dry seasons. Root damage weakens a plant's resistance to disease and makes ports through which pathogens can easily pass and take advantage of the plant's weakened condition. Leucaena seedlings are particularly susceptible to infection by the fungi Camptomeris leucaenae and Colletotrichum gloeosporoides, and the bacterium Pseudomonas sp. (Lenne, 1980).

Rice is an important food crop that is both direct-seeded and transplanted (Grist, 1975). The two methods of propagation produce vast differences in root morphology. During transplanting, the partial removal and destruction of a root system that is more or less already established alters the further development of the roots so that they are very fibrous and extend only into the uppermost layers of the soil. Direct-seeding of rice produces roots which extend much deeper and wider than in transplanted rice (Grist, 1975). The length, number, and weight of the roots per adult plant as well as the weight of the tops and the grain yield are higher in direct-seeded rice (Nagai, 1962).

One must weigh the advantages and disadvantages of transplanting. The seedlings destined for transplanting are given an additional growth period unrestricted by competition from other seedlings and weeds (Toumey and Korstian, 1957). Transplanting is highly recommended for many vegetable crops, most importantly in dry or temperate regions where the

growing season is limited (Thompson, 1939). Even though direct-seeding is often easier and cheaper than transplanting, environmental constraints may make transplanting the only feasible way to propagate some plants. Shalla and Doughton (1978) support the use of container nurseries to propagate Douglas fir seedlings because they allow a great deal of control over a seedling's environment and promote consistency in seedling growth.

D. Soil Nitrogen

The ready availability of nutrient elements in the growth medium is important in producing a healthy legume with a vigorous N_2-fixing symbiosis. Improper amounts of nutritional factors may limit N_2-fixation in the following ways: slowing development of free-living rhizobia in the rhizosphere, impairing formation of root nodules and growth of the host, and/or reducing nodule activities (Johnson et al., 1965; Edwards, 1977; Munns, 1977).

Nitrogen present in a combined form in the soil may affect the legume-*Rhizobium* symbiosis in a variety of ways depending on the form present. The application of NO_3^- inhibits nodule formation and growth while NH_4^+ inhibits nitrogenase synthesis and activity (Dart, 1977; Franco, 1977; Miller et al, 1982). Nodulation in *Pisum arvense* was reduced with NO_3^+ applications of 300 ppm (Edwards, 1977). However, small amounts of N have been shown necessary for symbiosis establishment, optimum growth, and high yields of legumes (Franco, 1977; Kang et al., 1977). Several studies have shown the beneficial effects of providing small quantities of N on a continuing basis during the growth of a

legume. Lotus pedunculatus grown with 50 to 250 ppm N responded with stimulated nodule development and nitrogenase activity (Pankhurst, 1981). In a pot experiment utilizing sand for growth medium, 11.7 ppm N in the form of urea inhibited aging of alfalfa nodules and prevented the lysis of the bacteroids in them (Strzelee, 1972). In the absence of combined N, the early growth of the legume is often slow because the development of the symbiotic system imposes a delay in growth and a significant energy cost on the young seedling (Haystead et al., 1980). Starter N may help a legume overcome this period of N stress prior to the commencement of N_2-fixation, especially under tropical conditions where the N demand is quite high during early development (Dart, 1977; Dilworth and McComb, 1977; Edwards, 1977). Stimulation of nodulation by the addition of small amounts of N during the 2 weeks following seedling emergence has been observed in soybeans (Kang et al., 1977). In a greenhouse experiment, Guss and Dobereiner (1972) found that two applications of 46 ppm mineral N to a podzolic soil increased the growth of field beans (Phaseolus vulgaris L.). In another greenhouse study, Gates and Wilson (1974) found that Stylosanthes humilis growing in an infertile solodic soil exhibited greater nodule dry weight and higher yields with a single application of 7 ppm N as NH_4NO_3.

E. Mycorrhizae

In addition to the symbiotic association with Rhizobium, the root systems of leucaena and other legumes also maintain an intimate relationship with a group of soil fungi. This root-fungus symbiosis is known as a mycorrhizae (Pirone, 1978). Common to the legumes are the endomycorrhizae which develop within the root and form intracellular hyphal

clusters (arbuscules), vesicles, and an extensive network of external hyphae (Mosse, 1977b; Ross, 1979). The presence of the arbuscules and vesicles in the endomycorrhizae cause the fungi to be termed vesicular-arbuscular (VA) endophytes (Atlas and Bartha, 1981).

The VA endophytes are not host-specific but certain endophytes may form preferential associations with specific host species (Mosse, 1977b). These endophytes are also obligate symbionts and depend on a living plant root for growth (Ross, 1979).

Many plant species forming an association with VA endophytes have been shown to assimilate P, K, NO_3^-, S, Zn, and perhaps Si much more readily than roots that are free of the fungus (Alexander, 1977a; Mosse, 1977b; Vander Zaag et al., 1979; Yost and Fox, 1979; Ojala and Jarrell, 1980). Mycorrhizal mycelia play a similar role to root hairs in that they aid water absorption by the plant (Taylor, 1962). The lack or presence of the proper mycorrhizal fungi may influence the survival and growth of exotic trees in a new environment (Goor and Barney, 1968). According to Mosse (1981), mycorrhizal fungi may have an important role in soil aggregation and hence may serve as a means of erosion control.

In soils containing very little available P, Hayman and Mosse (1971) recorded large increases in shoot dry weight for Coprosma and onions infected with mycorrhizae. As the nutrient content of the soil improves, a plant's reliance on the mycorrhizal symbiosis may decrease (Mosse, 1973). Barrows and Roncadori (1977) found that when cultural conditions were favorable for poinsettia (Euphorbia pulcherrima) the mycorrhizal symbiosis appeared to be of minimal importance. A leucaena study showed that P uptake was much greater in mycorrhizal leucaena (non-fumigated soils) than in non-mycorrhizal leucaena [fumigated soils (Yost and Fox,

1979)]. In this study, leucaena grown in a fumigated, low P soil were very deficient in P until fairly large amounts of P (1.6 ppm P in soil solution) were added to the soils. The reliance of leucaena on mycorrhizae decreased in the non-fumigated soils as the P levels were increased above 0.012 ppm P in soil solution. In addition to P, high levels of soil N may have negative effects on the mycorrhizal development and growth stimulation of legumes (Chambers et al., 1980; Safir and Duniway, 1982). Although N and P fertilizers can reduce mycorrhizal infection, they may increase it in very deficient soils by improving plant growth (Mosse, 1977b; Chambers et al., 1980).

Mycorrhizal infection occurs naturally in many soils, but it has been observed that inoculation with a proven inoculum can lead to additional growth increases (Mosse, 1977b and 1981). However, the benefits of inoculation are usually smaller in unsterile than in sterile soils because even the uninoculated plants in unsterile soil will eventually become mycorrhizal (Mosse, 1977a). Dependency on mycorrhizae varies with the plant species (Maronek et al., 1980). Some species, including L. leucocephala, are almost entirely mycorrhizal, at least during the early stage of seedling growth (Mosse, 1981). Although the infection is ultimately beneficial in low nutrient soils, mycorrhizal plants may experience a temporary growth depression that is similar to the initial depression caused by the formation of the Rhizobium nodules (Mosse, 1981; Linderman and Hendrix, 1982).

Endomycorrhizae have been shown to ease the stress of transplanting. Barrows and Roncadori (1977) reported that a mycorrhizal association with the fungus Gigaspora margarita reduced transplant shock in poinsettia grown under regimes of high temperature and low moisture. Substantial

increments in growth and reduced transplant injury have been recorded for both peach and avocado seedlings infected with mycorrhizal fungi (Gilmore, 1971; Menge et al., 1978). Post-transplanting dry weight yield of sugar maple, walnut, and green ash was improved with inoculation of the roots with a mixture of Glomus mosseae and Glomus etunicatus (Schultz et al., 1981). In a nursery study, Kormanik et al. (1977) demonstrated that the percentage of transplantable sweetgum (Liquidambar styraciflua L.) seedlings could be greatly increased if they were initially inoculated with G. mosseae. Mosse (1981) stressed the need to evaluate the potential use of mycorrhizal L. leucocephala transplants in erosion control on denuded sites in the tropics.

The tripartite association of legumes, Rhizobium, and mycorrhizal fungus has only been recently examined in detail to determine the extent of interactions between the components in maintaining a healthy plant. Since symbiotic N_2-fixation has a high P requirement, rhizobia in the nodules may benefit from a juxtaposition with mycorrhizae, especially if the P transported from the soil by the mycorrhizae is made immediately available to the nodules (Asimi et al., 1980). Smith and Daft (1977) and Barea et al. (1980) observed that mycorrhizal inoculation with G. mosseae stimulated nodulation in alfalfa. Asimi et al. (1980), reported that soybeans inoculated with G. mosseae nodulated better and had a higher nitrogenase activity. Also, improved P uptake, growth, and yield increases were noted in the mycorrhizal plants. Mycorrhizal alfalfa was reported to accumulate a higher percentage of N in plant tissues at several levels of added P (Mosse, 1981). Bethlenfalvay and Yoder (1981) grew soybeans inoculated with G. mosseae in perlite and supplied them with several levels of P in a nutrient solution. They found the presence

of mycorrhizae to benefit nodulation, acetylene reduction, and shoot weight most at an available P level of 0.62 ppm. Mosse (1977b) observed in several soils that Trifolium, Stylosanthes, and Centrosema inoculated with appropriate rhizobia failed to nodulate unless the plants were either inoculated with mycorrhizal fungi or given rock phosphate. French beans inoculated with mycorrhizae at planting produced nodules that were 10% heavier than those from plants treated with P (Daft and El-Giahmi, 1974).

III. MATERIALS AND METHODS

All experiments in this study, unless otherwise indicated, were conducted at the University of Hawaii in the Agronomy and Soil Science greenhouse #1, Manoa Valley, Honolulu. In this section, general listings of materials, procedures, and preliminary work are presented first. Then, formats of the main experiments are detailed.

A. General

1. Culture media and chemicals.

Yeast mannitol agar (YMA), as described by Vincent (1970), was used for growth of N_2-fixing Rhizobium on slants and culture plates. The YMA was used with or without streptomycin sulfate (500 ppm) plus demosan (50 ppm) and actidione (100 ppm). Streptomycin sulfate was added to the YMA to suppress bacterial contaminants and demosan and actidione were added to suppress fungal contaminants. These chemicals were employed for the development of an antibiotic-resistant mutant of Rhizobium and for strain recognition.

2. Preparation of cultures.

Rhizobium strain TAL582 (CB81) was obtained from the Niftal project of the Department of Agronomy and Soil Science, Maui, HI. Stock cultures were maintained on YMA slants at 4°C and were transferred every two months to fresh YMA slants to maintain culture viability. Working cultures were prepared whenever new stock cultures were made.

To follow the persistence of Rhizobium strain TAL582 in association with Leucaena leucocephala (Lam.) de Wit, antibiotic labelling of this

strain with streptomycin sulfate was employed (i.e., a streptomycin-resistant mutant was developed). A spontaneous mutant of TAL582 resistant to streptomycin was obtained by adding a 10 ml portion of a sterile Vincent's saline solution (Vincent, 1970) to a 7-day-old YMA slant of the bacterium. The culture was then scraped with an inoculating loop and the mixture was decanted into a sterile tube and shaken on a vortex mixer to disperse the bacterial suspension. Dilutions of the suspension (10^{-1} and 10^{-2}) were then prepared in Vincent's saline solution. One milliliter of a given dilution was added to a sterile petri plate. Sterile YMA (46°C) containing 500 ppm streptomycin sulfate was poured into the plates and thoroughly mixed with the culture suspension. The plates were incubated at 30°C until growth was apparent. The streptomycin-resistant culture (TAL582SR) was then tested for stability. For this purpose, Rhizobium colonies from the streptomycin sulfate-containing plates were inoculated onto YMA slants. After two subsequent transfers on streptomycin sulfate-free YMA slants at weekly intervals, serial dilutions of the culture were made in Vincent's saline solution and the number of colonies of the resistant culture growing on plates containing YMA with or without streptomycin sulfate were compared. Similar numbers on both media suggested that the mutants were stable in their resistance to streptomycin. Later, tests (M. Habte, personal communication) showed that TAL582SR was also resistant to 50 ppm demosan. Strain TAL582SR was maintained on YMA slants in the same manner as its wild parent, strain TAL582.

An experiment was done to determine if TAL582SR retained its ability to infect and fix N_2 as well as its wild parent, TAL582, in association with L. leucocephala (Lam.) de Wit (Appendix A). It was found that the

wild and mutant strain did not differ from each other with regard to the above concerns.

3. Medium for growing leucaena seedlings for transplanting.

The pre-transplanting medium in which the transplants were grown consisted of a 1:1 mixture (dry-weight basis) of moistened vermiculite and sphagnum peat moss treated with $CaCO_3$ (approximately 0.05 g $CaCO_3$/g medium) to bring the pH to approximately 7.0. The moistened mixture was sealed and set aside for a few days for the pH to stabilize. The medium was then sterilized by gamma irradiation from a ^{60}Co source (Food Science and Technology Laboratory, University of Hawaii). Sterilization of different batches of medium was performed on several occasions and the dosage applied ranged from 2 to 3 MRad. This was sufficient to ensure complete sterilization of the medium.

4. Preparation of soils for growth of direct-seeded leucaena and post-transplanting growth of the transplants.

The soil used was a vertic haplustoll (a very fine kaolinitic, isohypothermic clay -- a member of the Waialua clay series). It was obtained from leucaena plots at the University of Hawaii research facilities at Waimanalo. This soil was selected because it sustained healthy leucaena plants and provided an opportunity to evaluate Rhizobium strain TAL582SR in the presence of indigenous rhizobia populations. Soil was collected to a depth of 15 to 25 cm. The soil was passed through a 4 mm mesh screen and mixed thoroughly before storage in plastic bags. Several soil collections were made at different times but always within a few meters of the previous collection sites. Soil moisture at the time of collection ranged from 17.4 to 23.8%. No soil ammendments had been

made to the leucaena plots for at least 3 years prior to collection of the soil (J. Brewbaker, personal communication). The characteristics of both the pre-transplanting medium and the Waialua clay soil are presented in Table 1. The measurements were: pH (1:1 soil to distilled water), extractable P (modified Truog), cation exchange capacity, and exchangeable bases (U.S. Soil Survey Laboratory Staff, 1972). Plant-available P (Olsen P) was determined using the method of Watanabe and Olsen (1965). Total N and inorganic N (NO_3^- + NO_2^- + NH_4^+) were determined according to the procedures of Bremner (1965a, 1965b) using a semi-micro-Kjeldahl apparatus.

5. Containers for growing leucaena plants.

Two types of containers were used for growing leucaena plants in the greenhouse. White plastic dibble tubes (0.05 liter) were utilized for raising seedlings for transplanting. Black plastic pots (2.60 liters) were employed for direct-seeded plants and transplanted seedlings.

6. Fertilizer.

The N-free liquid fertilizer (NFLF) of Burton et al. (1972) was employed to supplement nutrients in both the dibble tube medium and soil. The NFLF was used at 1.5 times the recommended strength due to the fertilization regimen chosen in the experiments. Application rates and additional modification of the NFLF are discussed in Parts B and C of Materials and Methods.

7. Seed treatment.

Seeds of L. leucocephala (Lam.) de Wit var. K8 were obtained from Dr. J.L. Brewbaker of the Horticulture Department, University of Hawaii.

Table 1. Chemical characteristics of the growth media used.

Medium	pH	CEC	Exchangeable Bases				Truog P	Olsen P	Total N	Inorganic N
			Ca	Mg	Na	K				
		Meq/100 g					ppm			
Peat and vermiculite	7.0	50.1	21.0	7.6	0.5	0.3	60.3	-	-	42.0
Waialua clay	5.9	32.1	12.8	7.3	0.2	2.3	56.0	31.0	1620	311

Seeds of uniform size were scarified with concentrated H_2SO_4 for 10 minutes (Brewbaker and Hutton, 1979) to induce rapid and uniform germination. The scarified seeds were then washed several times with sterile distilled water containing $CaCO_3$(0.05 g/liter). The $CaCO_3$ served to neutralize residual acidity on the seed surfaces (measured by taking pH of the wash water).

8. Inoculation of leucaena seeds.

Two 1-week-old YMA slants of TAL582SR were scraped and mixed in 10 ml of Vincent's saline solution and applied to scarified leucaena seeds in sterile Dow Ziploc plastic bags (Dow Chemical Corporation). The seeds and bacterial suspension were well mixed to ensure a good coating of Rhizobium on the seeds. A finely ground peat sterilized with gamma irradiation was then mixed with the seeds to provide protection from desiccation. The peat-covered seeds were germinated for 2 days on moistened filter paper in sterile petri plates at room temperature and then planted.

Plate counts of bacterial colonies were done to determine the amount of viable Rhizobium cells present on the inoculated seeds after incubation for 2 days. Seeds inoculated with strain TAL582SR and coated with sterile peat were added to sterile Vincent's saline solution to obtain successive 10-fold dilutions. One milliliter portions of the dilutions were added to petri plates and melted YMA (46°C) was then poured and thoroughly mixed with the suspension. Upon solidification, plates were incubated (30°C) for 5 days. The number of viable Rhizobium per seed was recorded at 0, 1, 2, 7, and 14 days after inoculation. Two replicates were prepared on each day. This procedure was repeated with another

group of inoculated seeds at a later date and the average values for the two trials are recorded in Table 2. At 2 days, i.e., the time of planting, the average was in excess of 10^8 viable Rhizobium cells per inoculated seed.

9. Planting and transplanting of leucaena plants.

The 0.05 liter dibble tubes were filled with approximately 11 g (dry weight basis) of the sterile peat-vermiculite medium. Three or four inoculated seeds showing signs of germination were planted in each dibble tube and covered with approxiamtely 1 cm of medium. Fifteen days after planting, the seedlings were thinned to one plant per tube. At the time of transplanting, the leucaena seedlings were removed from the dibble tubes for transplanting using water under pressure from a garden hose. This procedure reduced the possibility of root injury and allowed the root and medium to be discharged from the tubes as an intact unit.

Pots in which leucaena were seeded or transplanted were lined with plastic bags and filled with approximately 2600 g of soil (dry weight basis). The plastic bags were slit at the bottom to allow adequate drainage. In the direct-seeded treatments, 15 to 20 inoculated seeds were planted approximately 1 cm below the soil surface. A large number of seeds was used to ensure the development of adequate numbers of healthy seedlings. Preliminary studies had shown a high incidence of seedling loss of direct-seeded plants to the fungal pathogens Rhizoctonia sp. and Phytophthora sp. (A. Martinez, personal communication). Direct-seeded plants were thinned after 4 weeks. Dibble sticks were used to make depressions in the potted soil in which the transplanted leucaena were placed.

Table 2. Number of viable Rhizobium cells persisting on inoculated seeds.

Days after inoculation	Number of Rhizobium cells/seed ($x10^8$)
0†	1.39
1	4.64
2	3.67
7	0.63
14	0.12

† The 0, 1, and 2 day treatments were incubated at room temperature (25°C) but the 7 and 14 day treatments were refrigerated at 4°C.

10. Statistics.

Most statistical analyses (ANOVA, GLM) and data plotting were done on the University of Hawaii IBM 370 computer. The Statistical Analysis System (SAS) package and literature were utilized (Helwig and Council, 1979; Freund and Littell, 1981; Lindsey and Douglas, 1981). All statistical significances reported in this work are based on the 5% level of probability unless otherwise indicated.

B. The Influence of Propagation Methods and Age of Transplanting on the Performance of Leucaena and Its N_2-fixing Symbiont

In this experiment, growth and N_2-fixation in four age groups of direct-seeded and transplanted leucaena were compared (total number of treatments = eight). Also, comparisons were made among the transplants so the best age to transplant L. leucocehala (Lam.) de Wit could be selected.

At 90, 62, 35, and 25 days before a predesignated transplanting date (19 Jan. 1982), leucaena seeds inoculated with Rhizobium strain TAL582SR were planted in dibble tubes containing a sterile peat-vermiculite mixture. At each of the above four dates, leucaena plants were started from inoculated seeds in pots containing the Waialua clay soil. Prior to transplanting, all leucaena plants were considered completely randomized for statistical purposes. After transplanting, the experiment was arranged in a split-plot design. Plant ages (25, 35, 62, and 90 days at transplanting) were considered as main plots and methods of propagation (direct-seeding and transplanting) were considered as subplots. Pots

were placed approximately 10 cm apart on the greenhouse benches. All potted plants were grown for an additional 72 days after transplanting and were harvested on 1 and 2 Apr., 1982.

At the time of planting, and on a weekly basis thereafter, the transplants were fertilized by drenching the medium with NFLF. The peat-vermiculite mixture in the dibble tubes was found to have a water holding capacity of about 290%. Thus, each dibble tube seedling had a capacity to retain almost 32 ml of water or NFLF when fertilized. For the macronutrients added in NFLF, this translates into 12.0 mg KCl, 3.0 mg $MgSO_4 \cdot 7H_2O$, 3.0 mg $CaSO_4 \cdot 2H_2O$, and 2.5 mg $Ca(H_2PO_4)_2 \cdot H_2O$ applied per dibble tube per week. The dibble tube medium was kept moist by additional watering on a daily basis. The direct-seeded plants were also fertilized at planting and on a weekly basis thereafter. However, instead of drenching the pots, 100 ml of NFLF was added to the pots each week. After transplanting, both transplanted and direct-seeded plants received 100 ml NFLF per week. Additional water was applied to the potted plants as needed.

At the time of transplanting and at the termination of the experiment, plants were collected and the following parameters were measured: shoot and root dry weight, shoot height, nodule number and nodule dry weight, acetylene reduction by nodulated roots, and total shoot and root N. These analyses were made on 6 and 10 replicates per treatment at transplanting and harvest, respectively. However, less replicates were used in determining total N concentrations in plants. Due to a shortage of dry matter at transplanting, plant material from the six replicates was divided into two groups of three replicates each and was analyzed for N as duplicates. At harvest, five replicates were randomly selected from

each treatment for N analysis. Regressions were run for these parameters and treatment means were compared with the least significant difference test (LSD). Also, extent of nodule occupancy (i.e., infection) by Rhizobium strain TAL582SR was determined. Two replicates for each treatment were made but statistical analyses were not done (discussed below).

Plants were harvested over a 2-day period, both at transplanting and at the termination of the experiment. On both occasions, the plants were harvested between 1000 and 1200 hours under partly sunny skies.

For the acetylene reduction assay test, roots and growth medium were gently removed from their containers as an intact unit and the roots were washed free of the medium with tap water. Few nodules were lost in this process. The plants were then taken to the laboratory and the roots were severed from the shoot at the root collar, i.e., a visible bulge separating the root from the shoot at the soil line. With nodules intact, the roots were placed into 500 ml jars with plastic screw-top lids fitted with a rubber septum. The plastic lids were fit onto the jars with Parafilm "M" to produce an airtight seal. Air was removed from the jars and replaced with acetylene to generate a 10% acetylene atmosphere. The roots were incubated for 1 hour at room temperature (23°). Autoclaved roots prepared the previous evening served as killed controls. To measure ethylene production, 2 ml samples were withdrawn from the jars and transferred to a 20 ml sealed test tube (after 2 ml air were removed) for storing the sample until analyzed with a Perkin-Elmer 3B gas chromatograph. The volume of gas injected into the gas chromatograph was 0.2 ml. Ethylene production per plant per hour was calculated by comparing sample peak heights with those obtained by injecting pure ethylene standards into the gas chromatograph. Specific nodule activity (nmoles

C_2H_4/g nodule dry weight per hour) was calculated from the acetylene reduction and nodule dry weight data.

Shoot height was measured from the cut root collar to the tip of the stem where the newest terminal leaves were forming. Shoot and root samples were then oven-dried at 70°C for 2 days and weighed. Subsequently, the shoot/root ratios were calculated from these data. The nodules were counted, dried, and weighed separately. Nitrogen concentrations in shoot and root samples were determined root samples according to the semi-micro-Kjeldahl method as outlined by Bremner (1965a). Before N was determined, the plant material was ground to pass through a 42 mesh (0.35 mm) screen. A 0.1 g sample was used for the digestion. Nitrogen uptake was calculated by multiplying the N concentration (mg/g) by the weight (g) of the shoots or roots.

Additional plants were collected from each treatment over a period of 4 to 6 days to determine the percent of nodules occupied by Rhizobium strain TAL582SR. Nodules were selected at random from leucaena roots and surface sterilized for 15 minutes in 0.1% $HgCl_2$ in 50% ethanol. The nodules were then washed six times in sterile distilled water and aseptically crushed in Multiwell tissue culture plates (24 wells/plate -- Becton, Dickinson & Co.) containing solid YMA or YMA containing streptomycin sulfate (500 ppm), demosan (50 ppm), and actidione (100 ppm). Also, nodules were crushed in cluster plates containing YMA and actidione (100 ppm) as an unspecific check for overall rhizobia viability. One nodule was crushed per well with bacterial exudate being spread evenly over the medium. The plates were incubated for 1 week at 30°C. Growth in each cell was rated positive or negative. Growth on the chemically-amended YMA suggested that the bacteria originated from the

inoculum strain TAL582SR. Lack of growth on this medium suggested that the nodule bacteria were indigenous soil rhizobia. Two plates comprised a replicate and two replicates were tested from each treatment. At the time of transplanting, the two youngest direct-seeded and all dibble tube treatment groups required five to eight plants to yield enough nodules for each replicate. In the older direct-seeded treatment groups, two plants were used to supply nodules for a single replicate. At harvest, each replicate consisted of randomly selected nodules from two plants.

C. The Influence of Pre-transplanting Treatments on the Early Post-transplanting Performance of Leucaena and Its N_2-fixing Symbiont

This experiment was done after a preliminary screening of several pre-transplanting treatments. Initially, the possibility of utilizing vesicular-arbuscular (VAM) mycorrhizal inoculation was explored. Four species of endomycorrhizae were rated for infection potential in dibble tube-grown leucaena (Appendix B). On the basis of this infection study, Glomus mosseae was chosen as the VAM inoculum for subsequent studies. In a larger preliminary experiment, treatments composed of a G. mosseae inoculation, 4.4 ppm starter N, a second Rhizobium inoculation at transplanting, and combinations of these treatments were compared (Appendix C). Based on the results presented in Appendix C, treatments were modified or eliminated. For example, it was found that a second Rhizobium inoculation with TAL582SR at transplanting did not bestow any advantages so it was eliminated from further consideration. The results also suggested the need for a fresher, more concentrated G. mosseae inoculum.

An additional dibble tube experiment evaluating a wider range of starter N applications was conducted before settling on slightly higher levels of N used in this present study (Appendix D).

The treatments employed here were: 1) G. mosseae inoculum -- (M), 2) uninoculated control -- (Cm), 3) 10 ppm starter N -- [N(10)], 4) 25 ppm starter N -- [N(25)], 5) G. mosseae inoculum plus 10 ppm starter N -- [MN(10)], 6) G. mosseae inoculum plus 25 ppm starter N -- [MN(25)]. Freshly harvested propagules (spores, sporocarps, and infected root segments) of G. mosseae from the rhizospheres of corn plants were obtained in a water suspension from the laboratory of Dr. C. Murdock of the Horticulture Department. The suspension was refrigerated for 1 week until planting. The propagules were then further diluted with water. One milliliter aliquots were applied to dibble tubes containing the sterile peat-vermiculite medium. Leucaena seeds inoculated with TAL582SR were placed on top of the mycorrhizal inoculum and covered with medium. Each tube received greater than 170 spores and sporocarps of G. mosseae. The uninoculated control (TAL582SR inoculum only) and the nonmycorrhizal N treatments received 1 ml aliquots of the filtrate obtained by passing the mycorrhizal inoculum suspension through a 5 um Millipore filter. Nitrogen (10 and 25 ppm) was applied as NH_4NO_3 1 week after planting.

The seeds were planted on 24 May 1982 and grown for 4 weeks before transplanting on 21 June 1982. The seedlings in each treatment were arranged in the randomized complete block design (pots spaced 15 cm apart). Plants were sampled for analysis at transplanting, 4 weeks after transplanting (20 July 1982), and 8 weeks after transplanting (16 Aug. 1982).

All treatments were drenched with NFLF alone or with starter N 1 week after planting. The P level in the NFLF was reduced from 18 to 1.5 ppm P

to minimize the suppression of mycorrhizal infection. Subsequent nutrient drenchings with the low P NFLF were made twice a week until the time of transplanting. Thereafter, 100 ml of the low P NFLF was added to each pot once a week.

Shoot and root dry weight, shoot height, nodule number and nodule dry weight, and total shoot and root N were determined as before. Counts of the number of expanded compound leaves were made at 4, 6, and 8 weeks after transplanting. Nutrient determinations of shoots and roots collected 8 weeks after transplanting were made by the University of Hawaii Cooperative Extension Service using a Applied Research Laboratories (ARL) vacuum X-ray quantometer. For determination of mycorrhizal infection, fine roots were cut into segments approximately 1 cm in length and stained according to the methods of Phillips and Hayman (1970). Roots were soaked overnight in clearing and staining solutions instead of autoclaving. Fifty randomly selected stained root segments from each replicate were examined for infection in an open petri plate with a stereo dissecting microscope to determine the percentage of infected segments (Chambers et al., 1980; Giovannetti and Mosse, 1980). Each stained segment was examined for the presence or absence of arbuscules, vesicles, and/or spores. The number of replicates analyzed for each treatment varied with the parameters that were measured above and the time of measurement. For ease of presentation, replicate numbers are discussed in the Results section. Treatment means were compared using Duncan's multiple range test (DMRT).

IV. RESULTS

A. The Influence of Propagation Methods and Age of Transplanting on the Performance of Leucaena and Its N_2-fixing Symbiont

This experiment was conducted to compare transplanting and direct-seeding methods of leucaena propagation and to determine the influence of seedling age at transplanting on the early post-transplanting performance of leucaena and its Rhizobium symbiont. The results are presented in Figures 1 through 23. The $LSD_{0.05}$ bars in each figure are for comparisons between methods of propagation at a given age. In addition to the figures in the text, a tabular version of the data with appropriate $LSD_{0.05}$ and C.V. values are presented in Appendix E. The four age treatments resulted in plants that were 25, 35, 62, and 90 days old at transplanting. At harvest, these plants were 97, 107, 134, and 162 days old, respectively. The treatments will occasionally be referred to in the text by the age in days when transplanted (25, 35, 62, or 90 days) in combination with the method of propagation (direct-seeded = DS or transplants = T).

1. Performance at transplanting

At the time of transplanting, shoot dry weight for direct-seeded leucaena increased significantly with age in plants older than 35 days (Fig. 1). Shoot dry weight of the dibble tube seedlings (transplants) did not differ significantly with age. Beyond 35 days of age, the direct-seeded leucaena accumulated significantly more shoot dry weight than correspondingly-aged transplants. The 90-day-old transplants appeared less healthy than the other plants and showed incipient signs of chlorosis and leaf loss.

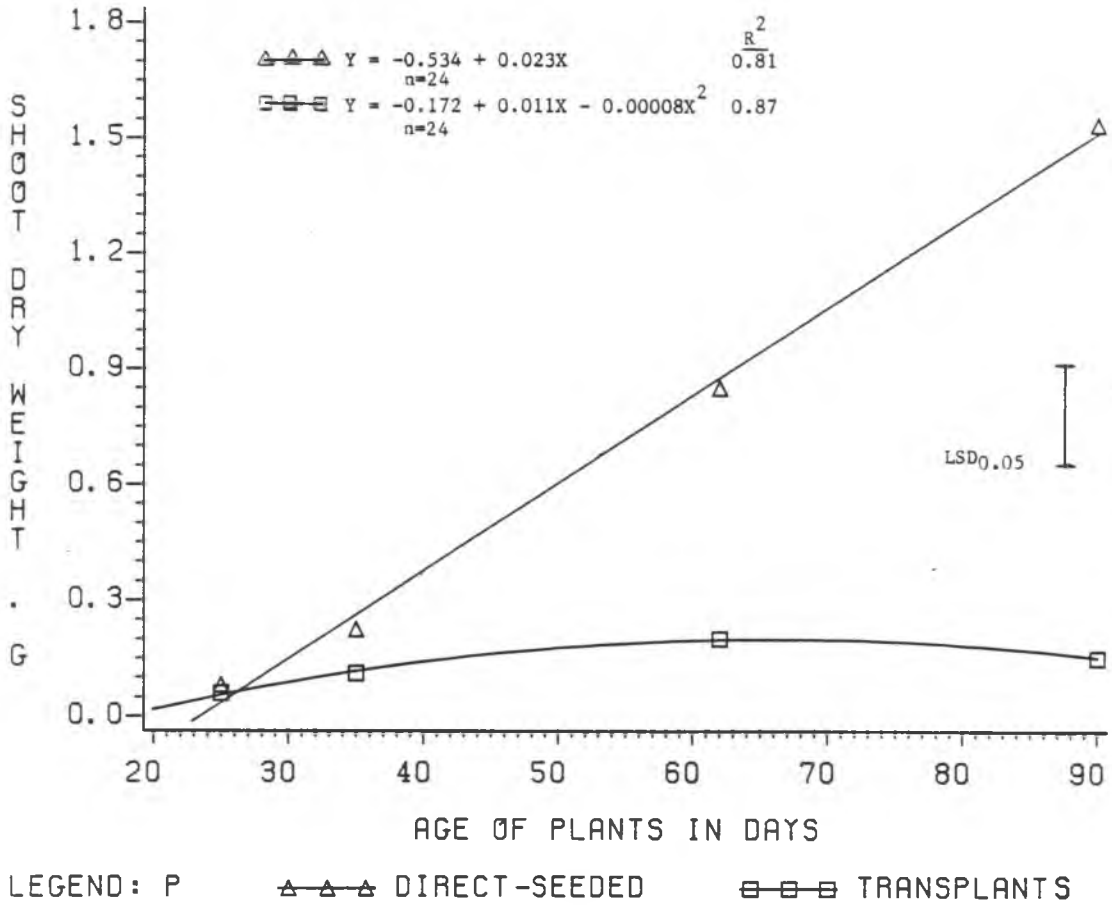

Fig. 1. Shoot dry weight at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

The root dry weights followed a trend similar to that for the shoot dry weights (Fig. 2). Root dry weight for direct-seeded leucaena increased significantly with age in plants older than 35 days. Age effects were not significant for the transplants. The direct-seeded leucaena accumulated significantly more root dry matter than the transplants at 62 and 90 days of age. Root development of the 25- and 35-day-old transplants was insufficient to allow the removal of the growth medium and roots as a single unit during transplanting. Transplants aged 62 and 90 days had more extensive root systems and were easily removed from the dibble tubes with the growth medium intact.

The shoot/root ratios decreased with age for both transplants and direct-seeded leucaena (Fig. 3). The values were significantly greater for the 25- and 35-day-old plants than for the 62- and 90-day-old plants. For any given age, differences between methods of propagation were not significant.

Shoot heights of 62- and 90-day-old direct-seeded plants were significantly greater than those of 25- and 35-day-old direct-seeded plants (Fig. 4). No significant differences in shoot heights due to age were observed in the transplants. Direct-seeded leucaena of 35, 62, and 90 days of age were significantly taller than correspondingly-aged transplants.

Root nodule number per plant of the direct-seeded leucaena was significantly greater for the 90-day-old plants than for all other ages (Fig. 5). There was no significant effect of age on nodule number for the transplants. Direct-seeded plants at 90 days of age had significantly more nodules than did 90-day-old transplants. The transplants had about 10 nodules per plant. All root nodules examined from the 25-, 35-, and

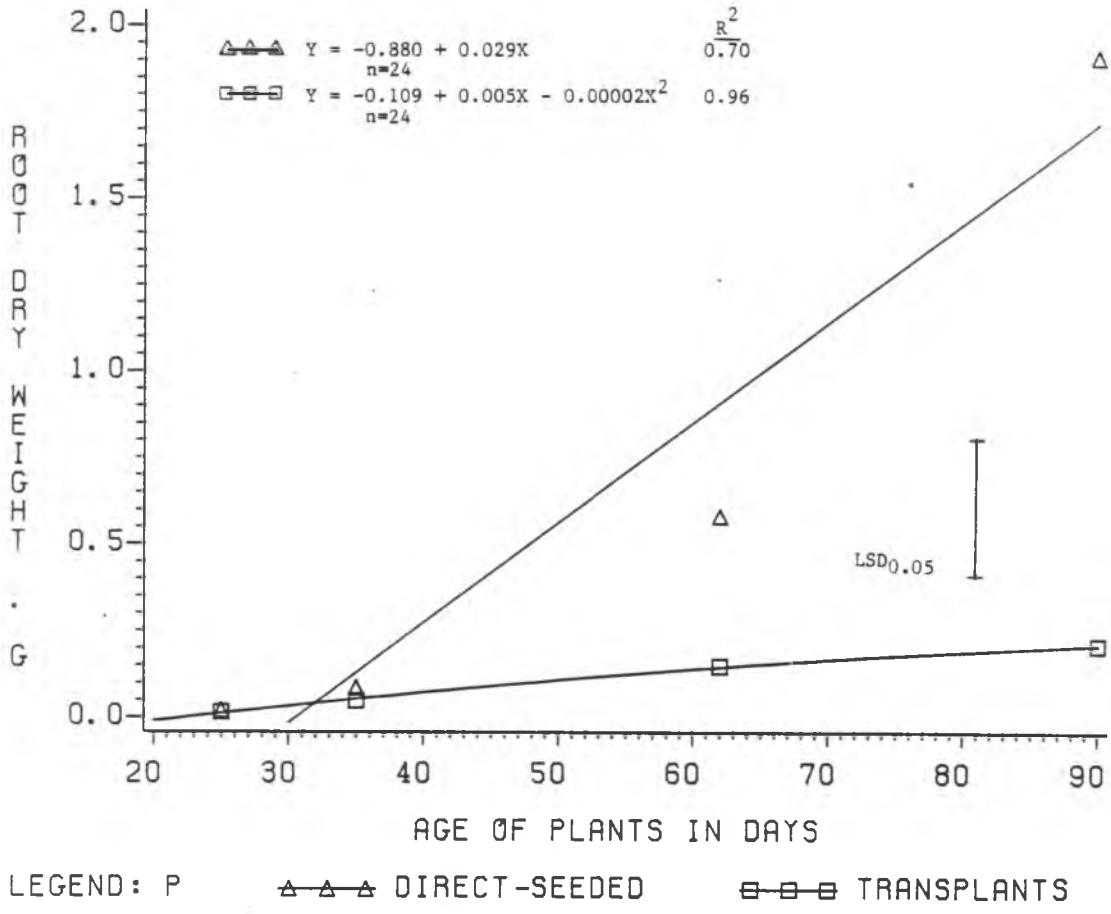

Fig. 2. Root dry weight at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

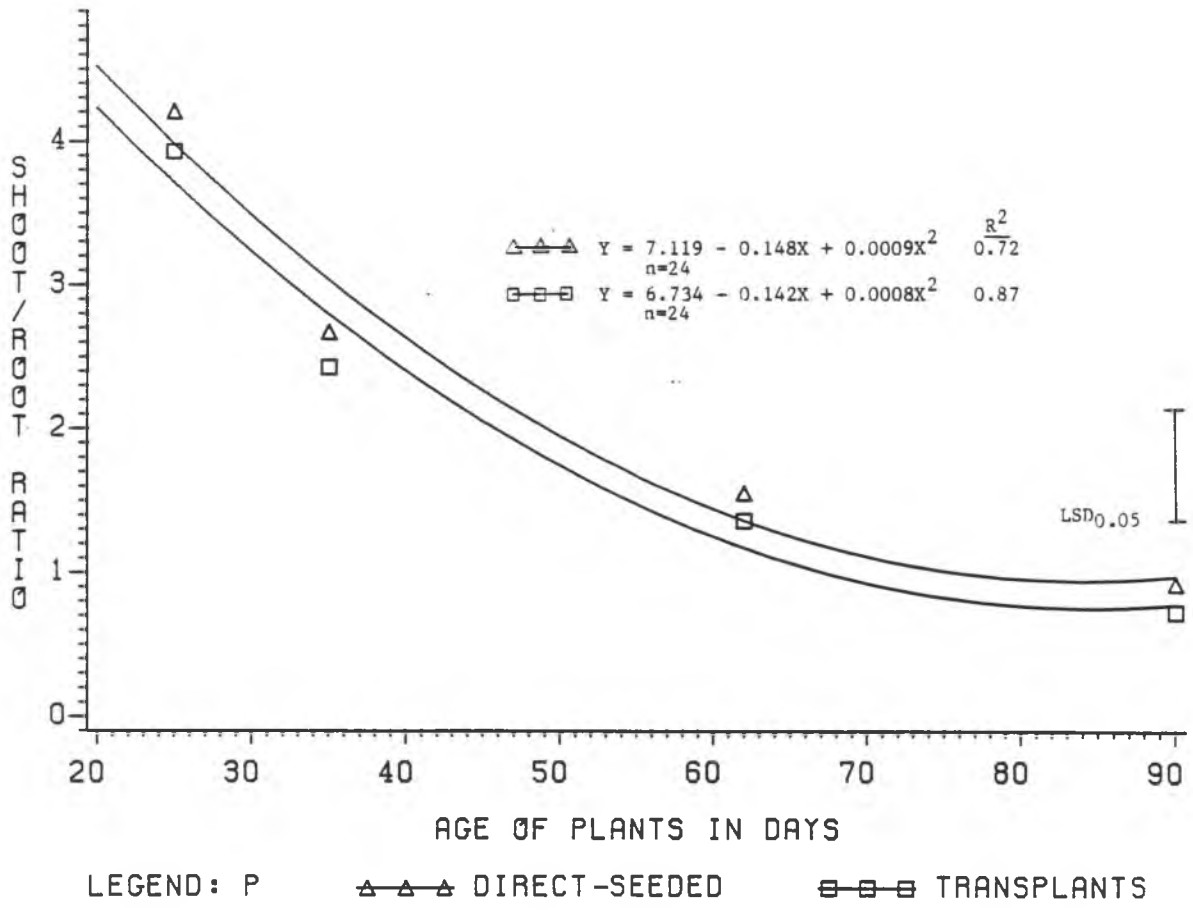

Fig. 3. Shoot/root ratios at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

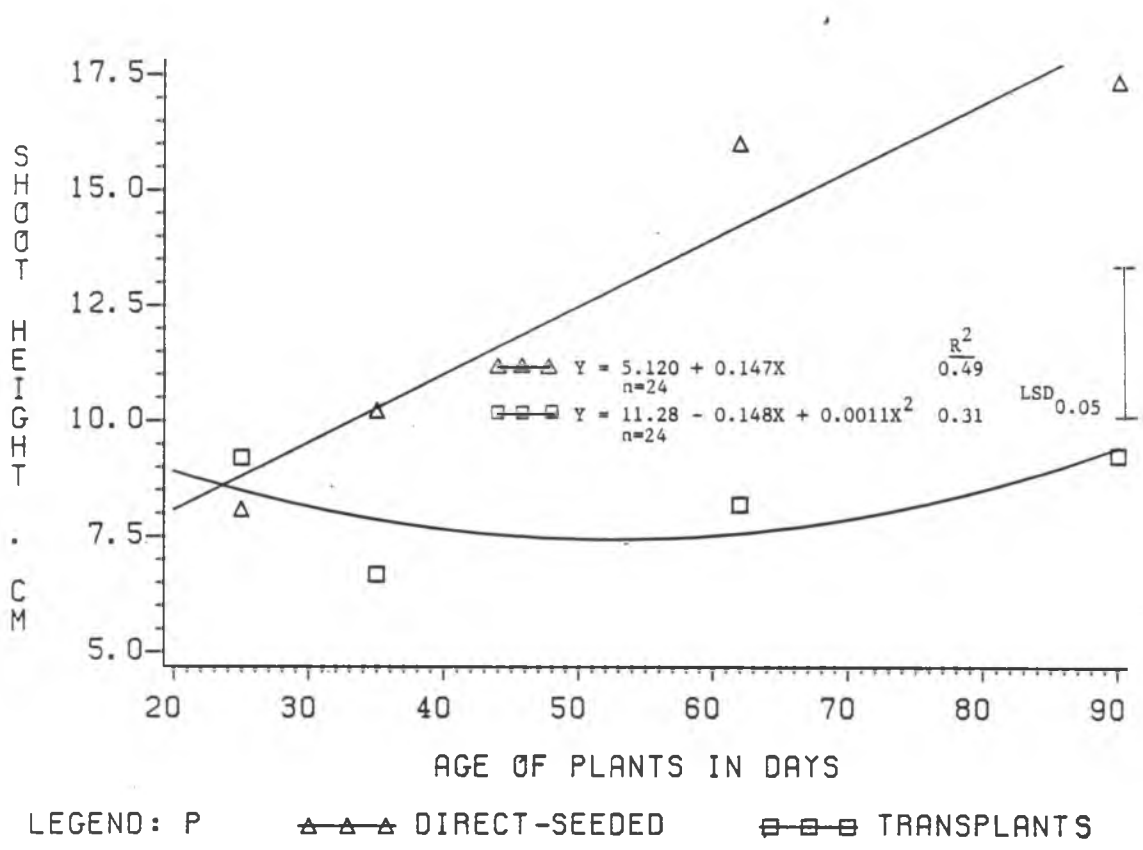

Fig. 4. Shoot height at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

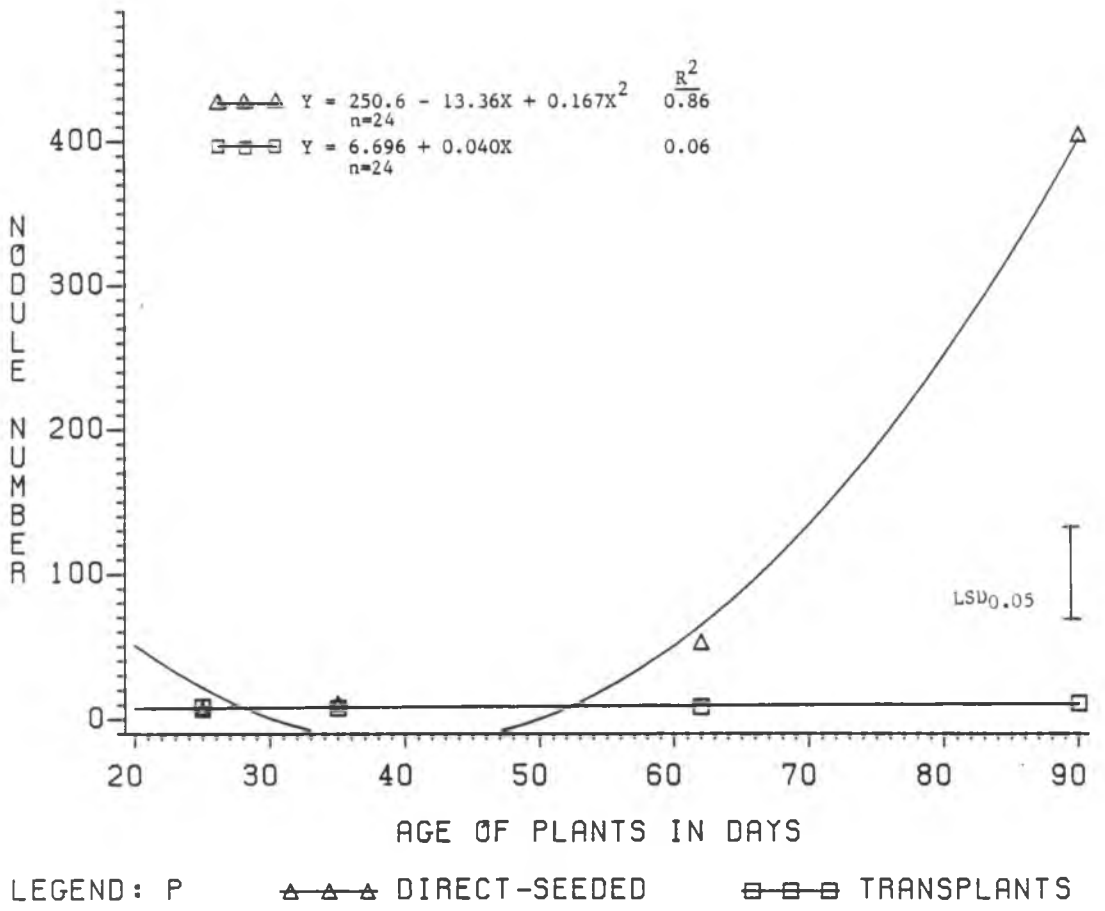

Fig. 5. Nodule number per plant at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

62-day-old transplants were effective as indicated by their red interiors. However, there was evidence of nodule decay on the 90-day-old transplants. Random examination of nodules from direct-seeded leucaena showed most had red interiors. As with the transplants, some decaying nodules were found in the root systems of the 90-day-old direct-seeded plants. Nodule breakdown in the older plants suggests that the average lifespan of a leucaena nodule may be between 2 to 3 months.

Nodule dry weights per plant of 62- and 90-day-old direct-seeded leucaena were greater than for younger direct-seeded plants (Fig. 6). Nodule dry weights of the transplants did not differ significantly from each other with increasing age. Sixty-two and 90-day-old direct-seeded plants accumulated significantly more nodule dry weight than transplants of the same age.

The shoot N concentration decreased significantly with increasing age for direct-seeded leucaena (Fig. 7). The twenty-five day old transplants had significantly higher N concentrations than the older transplants. The 25-, 35-, and 62-day-old direct-seeded plants had significantly higher N concentrations than transplants of the same age.

The root N concentration decreased with age for both transplants and direct-seeded plants (Fig. 8). Thirty-five-day-old direct-seeded plants had significantly higher levels of root N than the transplants of the same age.

Total N uptake by the shoots of direct-seeded leucaena increased significantly with age in plants older than 35 days (Fig. 9). Shoot N uptake in the transplants did not differ significantly with age. Direct-seeded plants of 35, 62, and 90 days of age accumulated significantly more shoot N than the correspondingly-aged transplants.

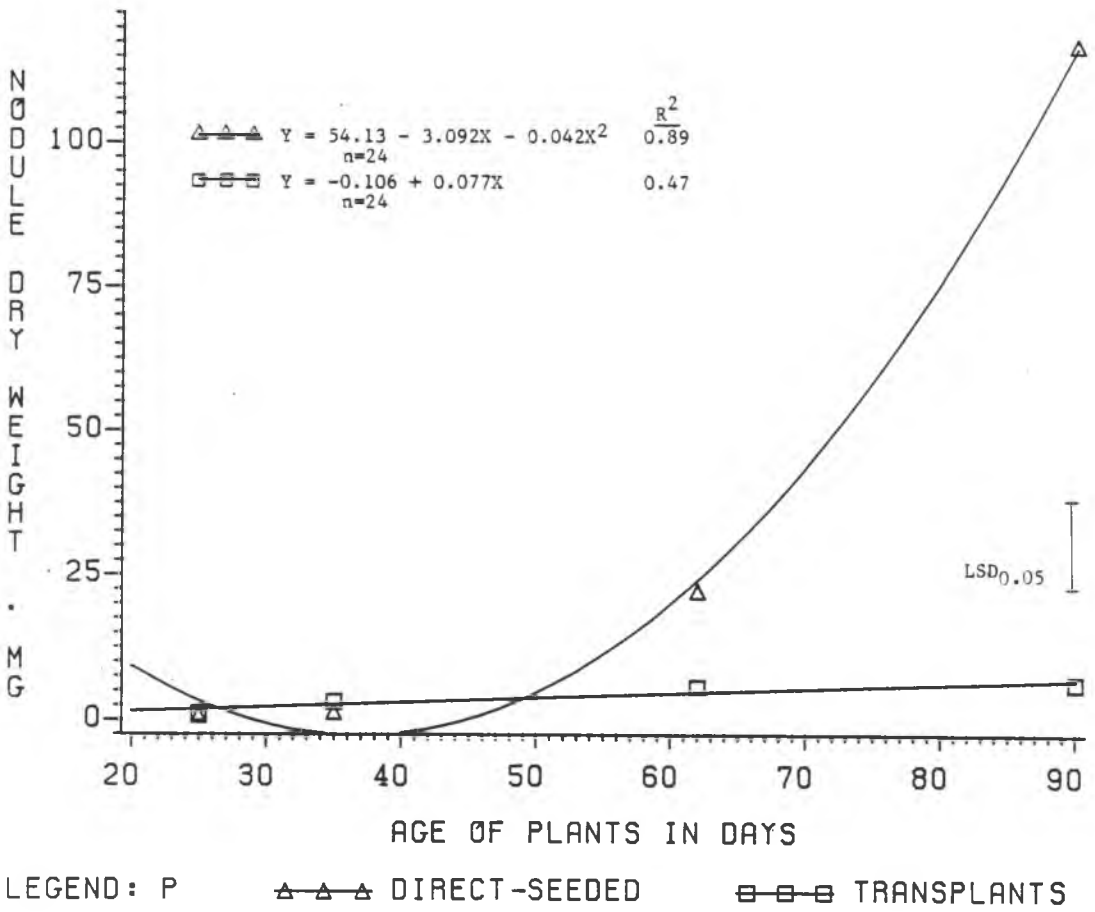

Fig. 6. Nodule dry weight per plant at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

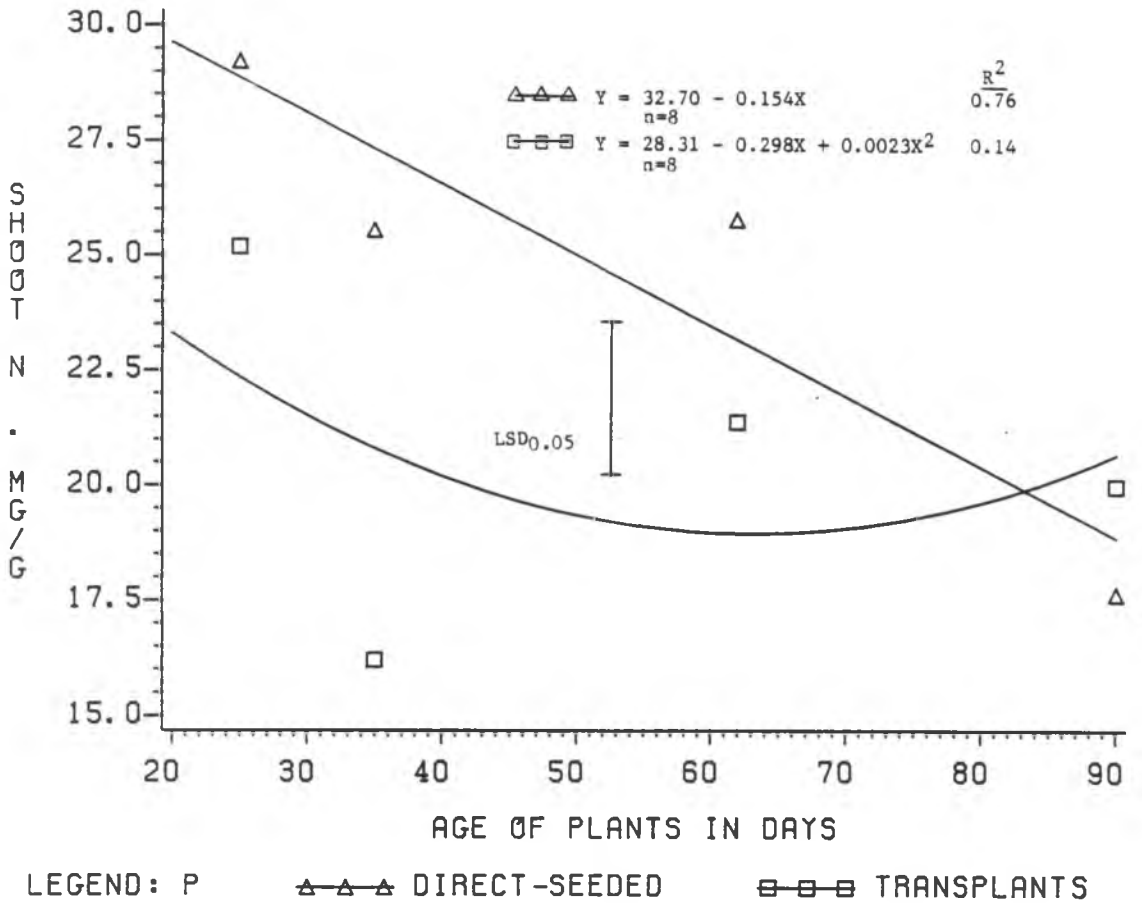

Fig. 7. Shoot N concentrations at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

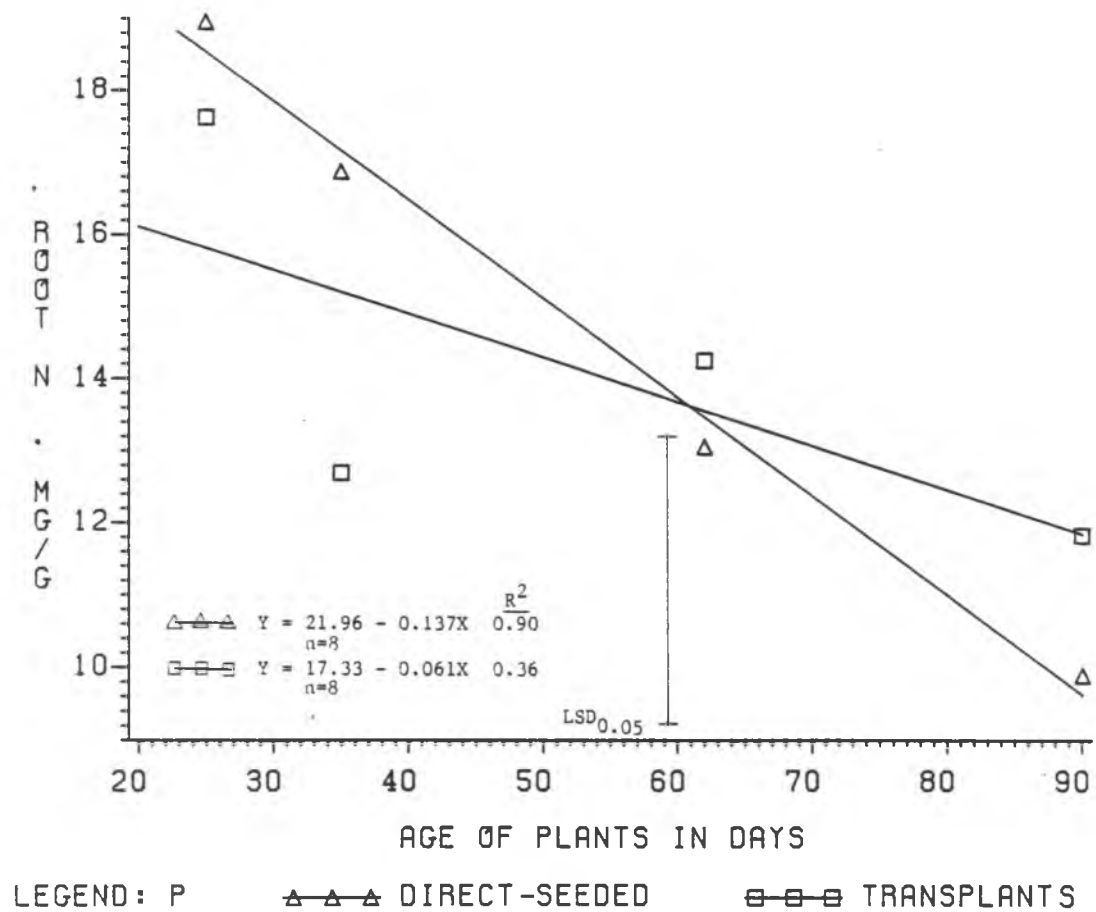

Fig. 8. Root N concentrations at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

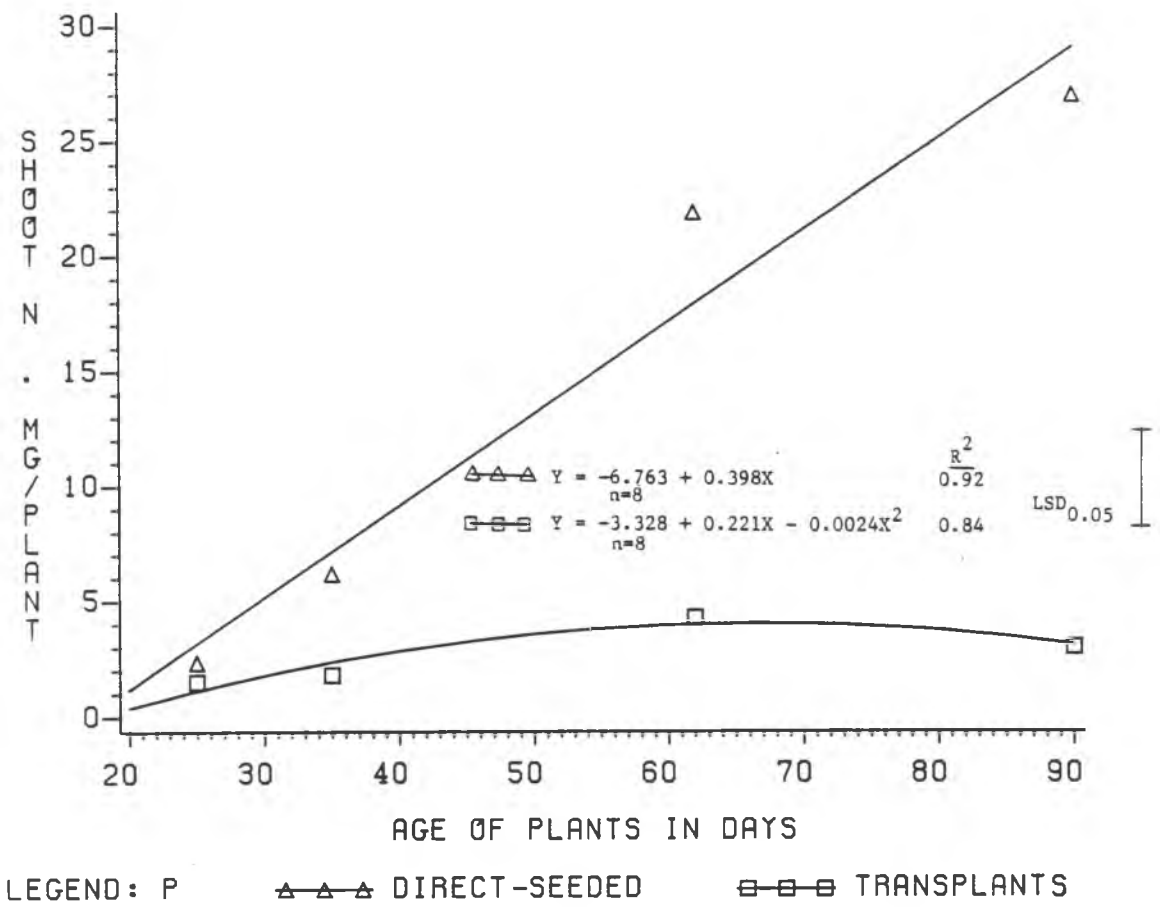

Fig. 9. Shoot N uptake at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

Total N uptake by the roots of direct-seeded leucaena increased significantly with age in plants older than 35 days (Fig. 10). Sixty-two- and 90-day-old transplants assimilated a greater amount of root N than the 25- and 35-day-old transplants. With regard to the method of propagation, 62- and 90-day-old direct-seeded leucaena plants accumulated a significantly greater amount of root N than transplants of the same age.

2. Performance at harvest

Leucaena plants that were 25, 35, 62, and 90 days of age at transplanting were now 97, 107, 134, and 162 days of age, respectively. With respect to shoot dry weight, 162-day-old direct-seeded leucaena (90DS) yielded significantly more shoot dry matter than 97-day-old direct-seeded plants [25DS (Fig. 11)]. No other differences were noted between the direct-seeded plants. The two older groups of transplanted leucaena produced significantly greater amounts of shoot dry matter than the younger transplants. For all ages, shoot dry weights of the transplants did not differ significantly from their direct-seeded counterparts.

The trend for root dry weights was somewhat different from that observed for shoot dry weights (Fig. 12). Direct-seeded plants aged 134 and 162 days (62DS, 90DS) produced significantly larger amounts of root material than the younger direct-seeded plants. Plants in the 25DS and 35DS treatments were not significantly different from each other in this regard. Transplants aged 134 and 162 days of age (62T, 90T) accumulated significantly greater amounts of root dry matter than younger transplants. With regard to comparisons between methods of propagation, 134- and 162-day-old direct-seeded plants (62DS, 90DS) accumulated a significantly greater amount of dry root matter than the transplants of

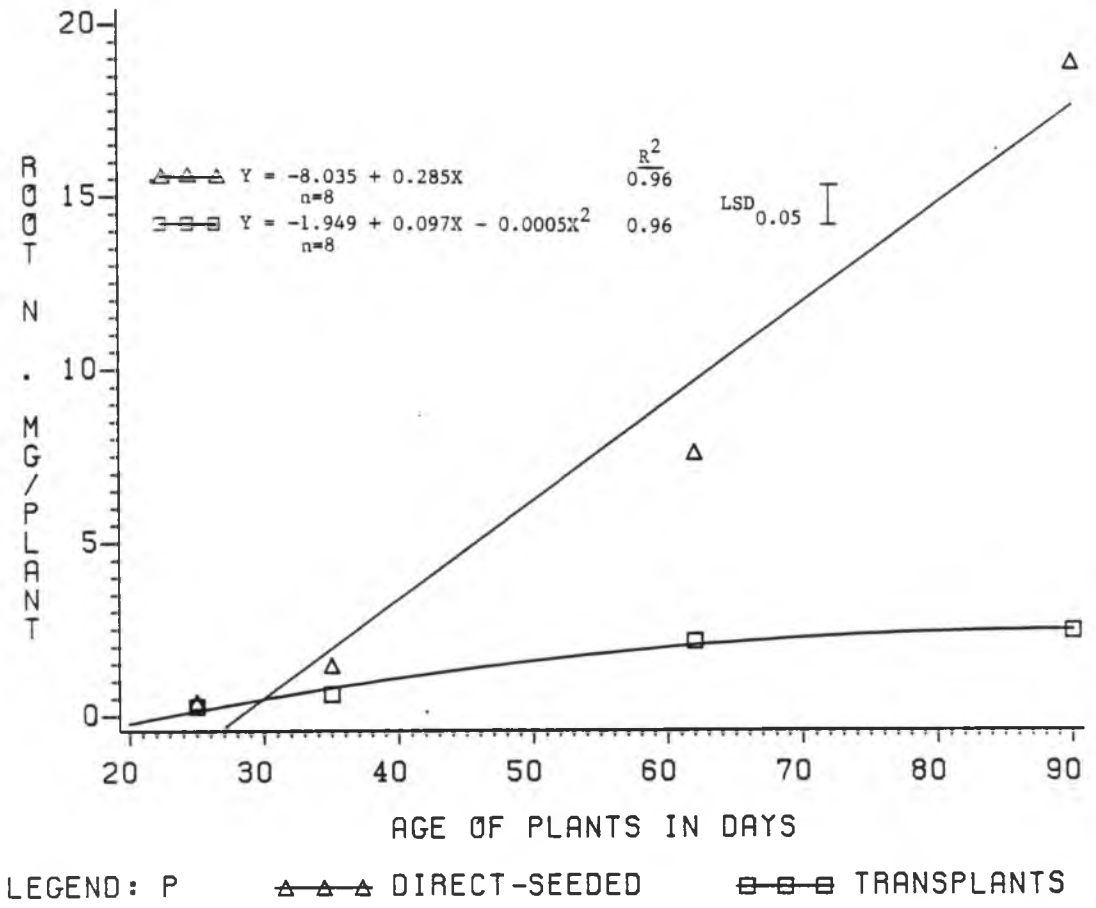

Fig. 10. Root N uptake at transplanting for leucaena started in dibble tubes (TRANSPLANTS) and soil (DIRECT-SEEDED).

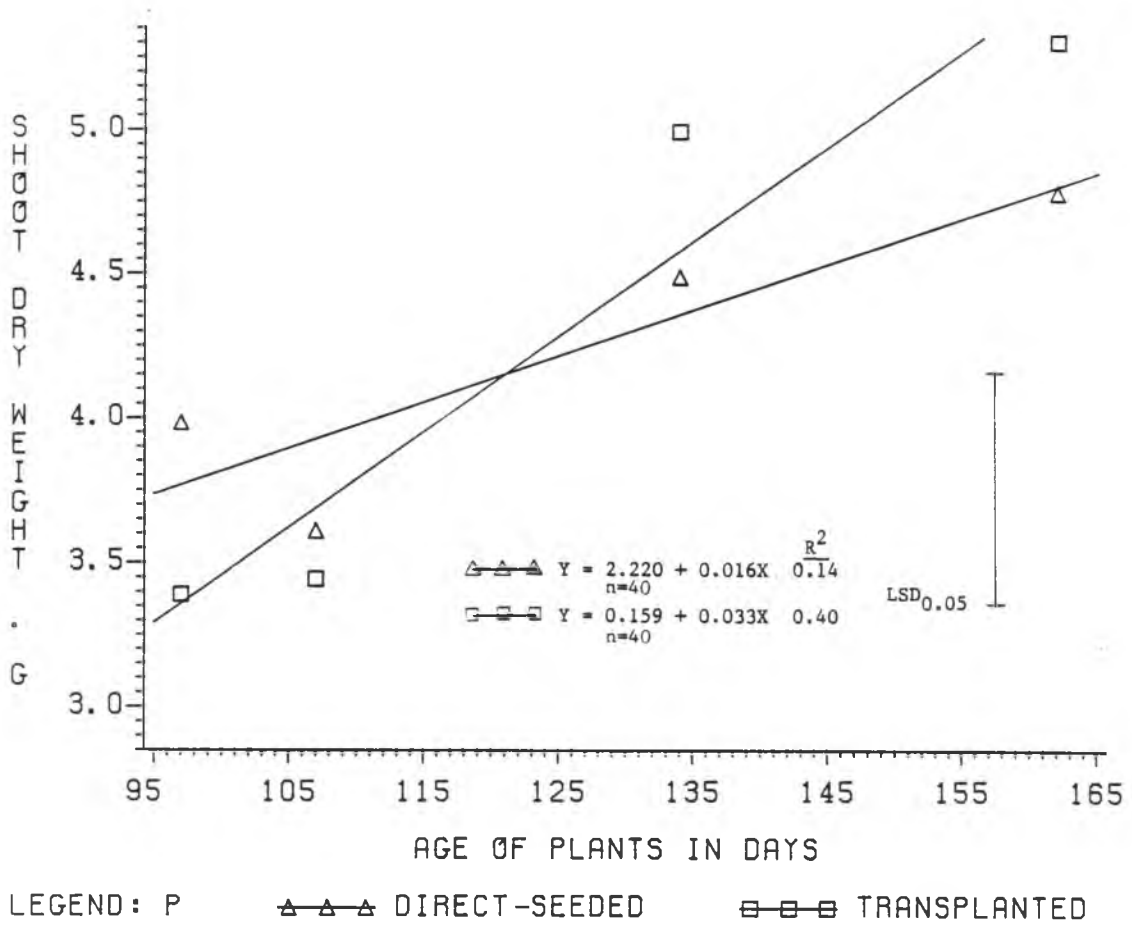

Fig. 11. Shoot dry weight at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

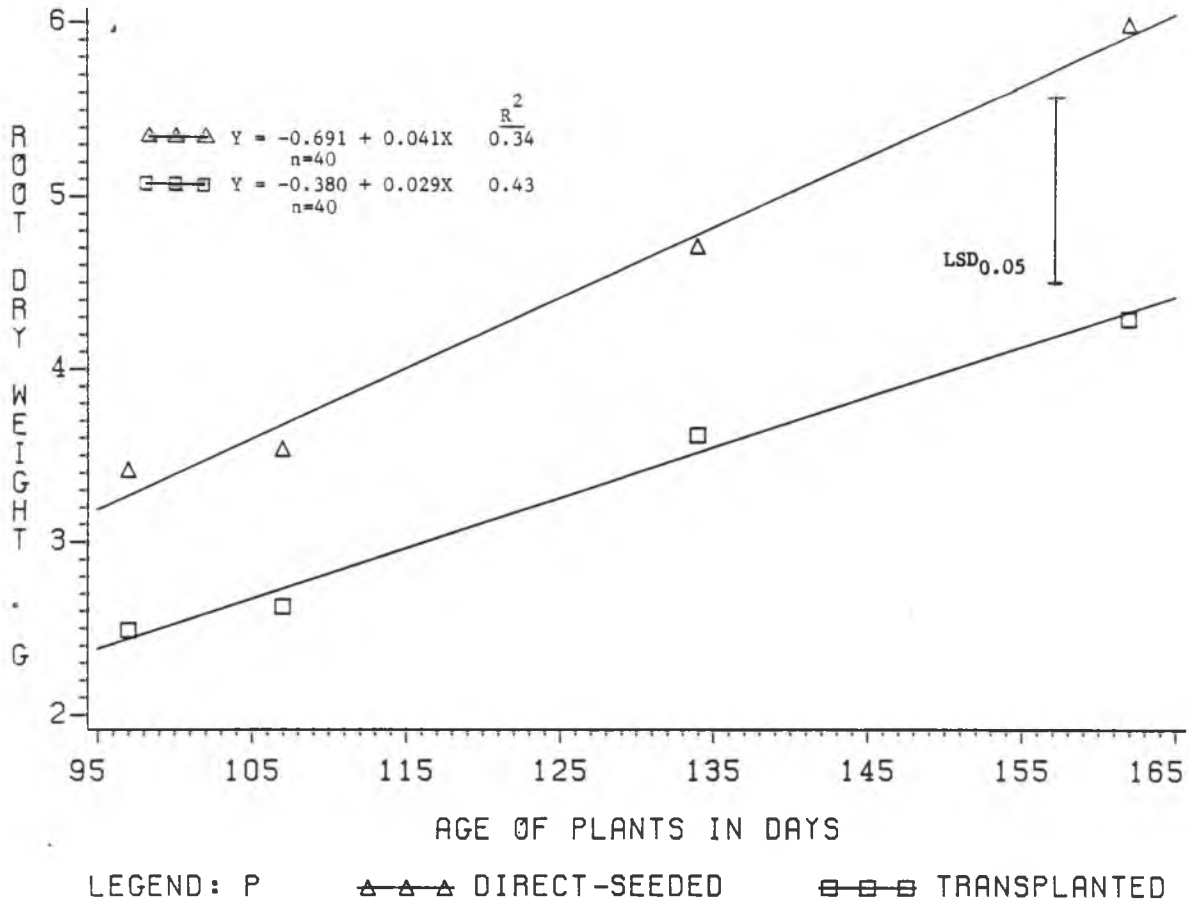

Fig. 12. Root dry weight at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

similar age. Root dry weight differences between transplants and direct-seeded leucaena were not significant at the two younger ages. Similarities in shoot growth and differences in root growth between direct-seeded and transplanted leucaena are illustrated in Fig. 13 for 107-day-old transplants (35T) and their direct-seeded counterparts (35DS). Examination of the roots of 134- and 162-day-old direct-seeded leucaena plants (62DS, 90DS) showed that they were starting to crowd the walls of the pots. Transplanted leucaena from all age treatments showed at least some regrowth of the taproot beyond where they had been air-pruned in the dibble tubes. In both transplanted and direct-seeded leucaena plants, vertical roots tended to spiral considerably when they reached the bottom of the pot.

Direct-seeded plants of 162 days of age (90DS) had significantly lower shoot/root ratios than 97- and 134-day-old direct-seeded plants [25DS, 62DS (Fig. 14)]. In the transplanted leucaena, treatment differences in the shoot/root ratios were not apparent. However, transplants of 107, 134, and 162 days of age (35T, 62T, 90T) had significantly larger shoot/root ratios when compared to their direct-seeded counterparts.

The shoot heights of 107-, 134-, and 162-day-old transplants (35T, 62T, 90T) were significantly greater than those of direct-seeded leucaena of the same age (Fig. 15). A significant age x method of propagation interaction was observed for this parameter. The interaction indicates that the response of plant height to age varied with method of propagation, i.e., the rate of increase in height with age was greater in the transplants.

Fig. 13. Root growth at harvest for direct-seeded and transplanted leucaena. Transplanting was done at 35 days of age.


35DS
35T

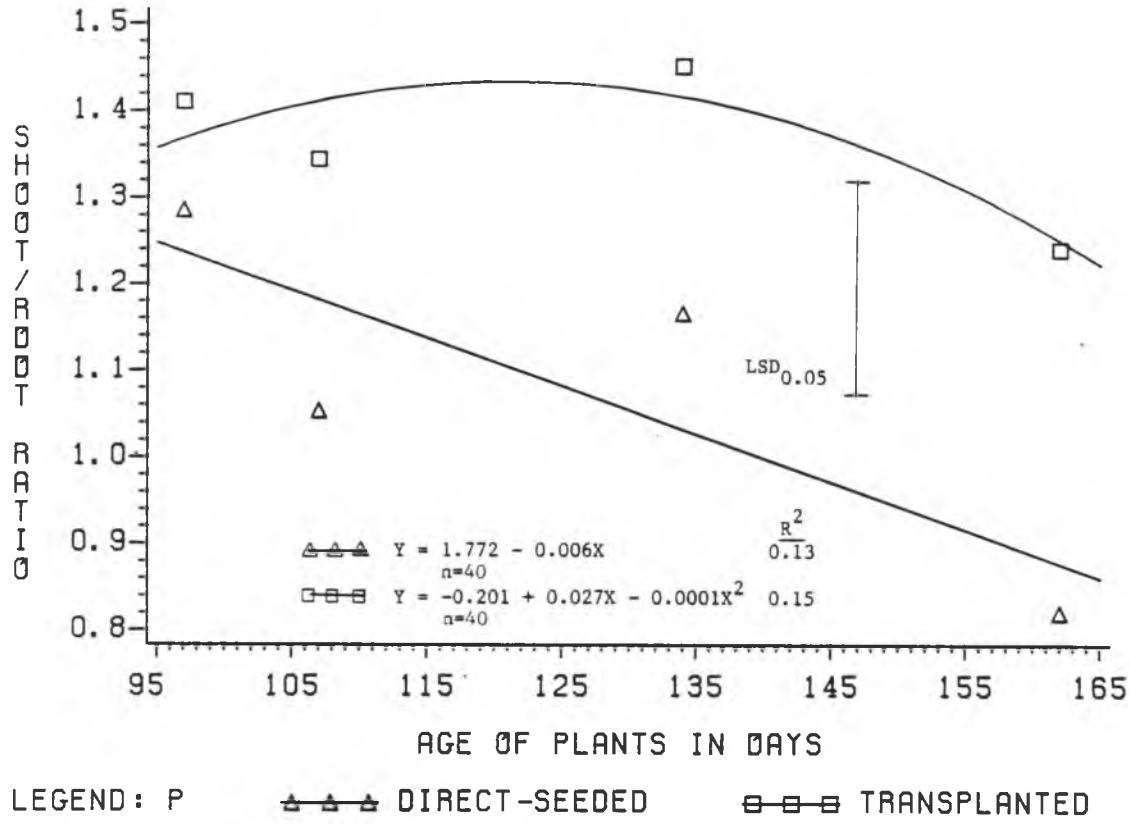

Fig. 14. Shoot/root ratios at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

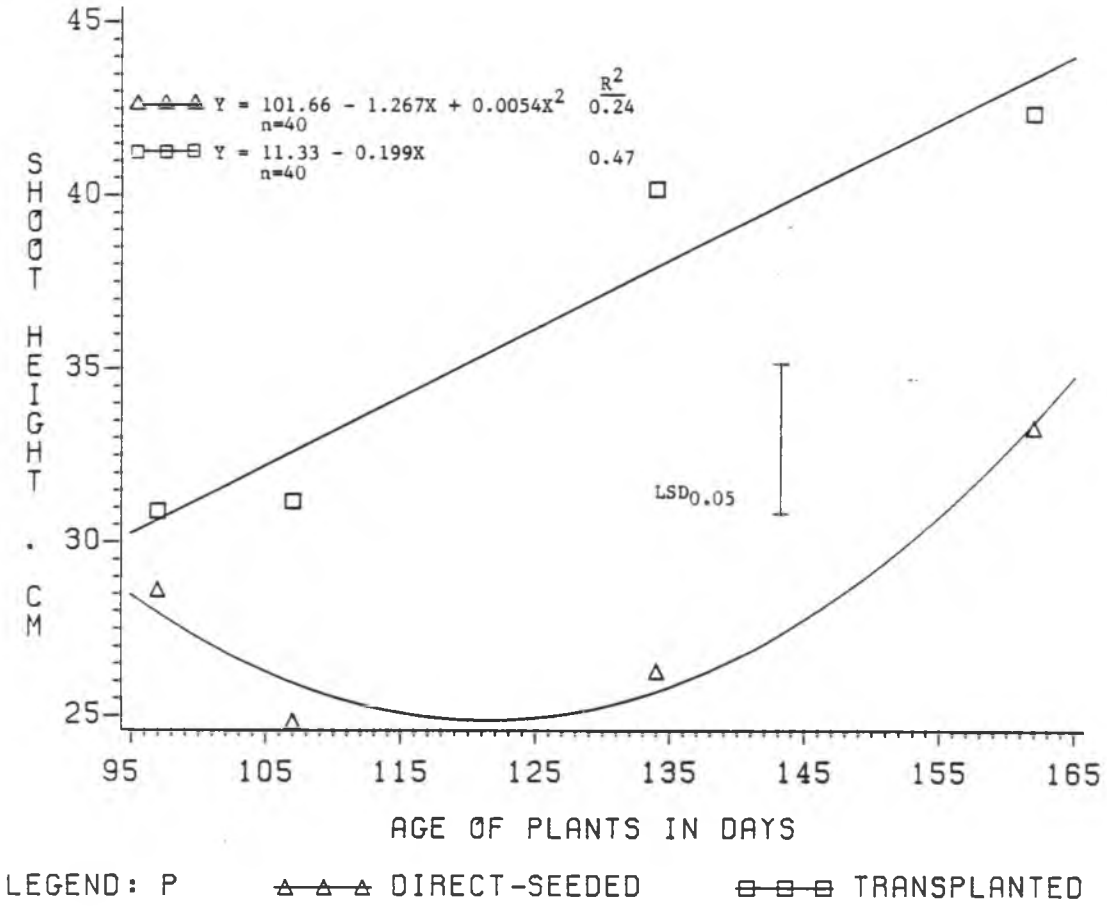

Fig. 15. Shoot height at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

With regard to the number of nodules produced per plant, direct-seeded plants had a significantly greater number than transplants of the same age (Fig. 16). There was a significant age x method of propagation interaction for root nodule number at this final harvest. At this time, most root nodules sampled at random had red interiors.

Nodule dry weights in direct-seeded plants was significantly higher than in similarly-aged transplants (Fig. 17). However, the average dry weights per nodule were 32%, 53%, 12%, and 45% higher, respectively, for 97-, 107-, 134-, and 162-day-old transplants (25T, 35T, 62T, 90T). The age x method of propagation interaction was significant. Correlation coefficients for nodule number and nodule dry weight were significant at transplanting ($r = 0.99$) as well as at harvest ($r = 0.98$). Similarly, nodule dry weight and root dry weights were significantly correlated at transplanting ($r = 0.97$) and harvest ($r = 0.84$).

Acetylene reduction was measured at both transplanting and harvest. Unfortunately, concentrations of ethylene (C_2H_4) produced at transplanting were so low that they fell below the linear range of the detector employed with the gas chromatograph and could not be measured accurately. Therefore, only the data procured at harvest are given here. Ethylene production in direct-seeded leucaena did not vary significantly with age (Fig. 18). Significantly higher C_2H_4 production was observed for 134- and 162-day-old transplants (62T, 90T) as compared to the younger transplants. Even though transplanted leucaena had fewer nodules and lower nodule dry weights than direct-seeded plants, C_2H_4 production was significantly higher for 134- and 162-day-old transplants (62T, 90T) than for direct-seeded plants of the same age. Ethylene production by

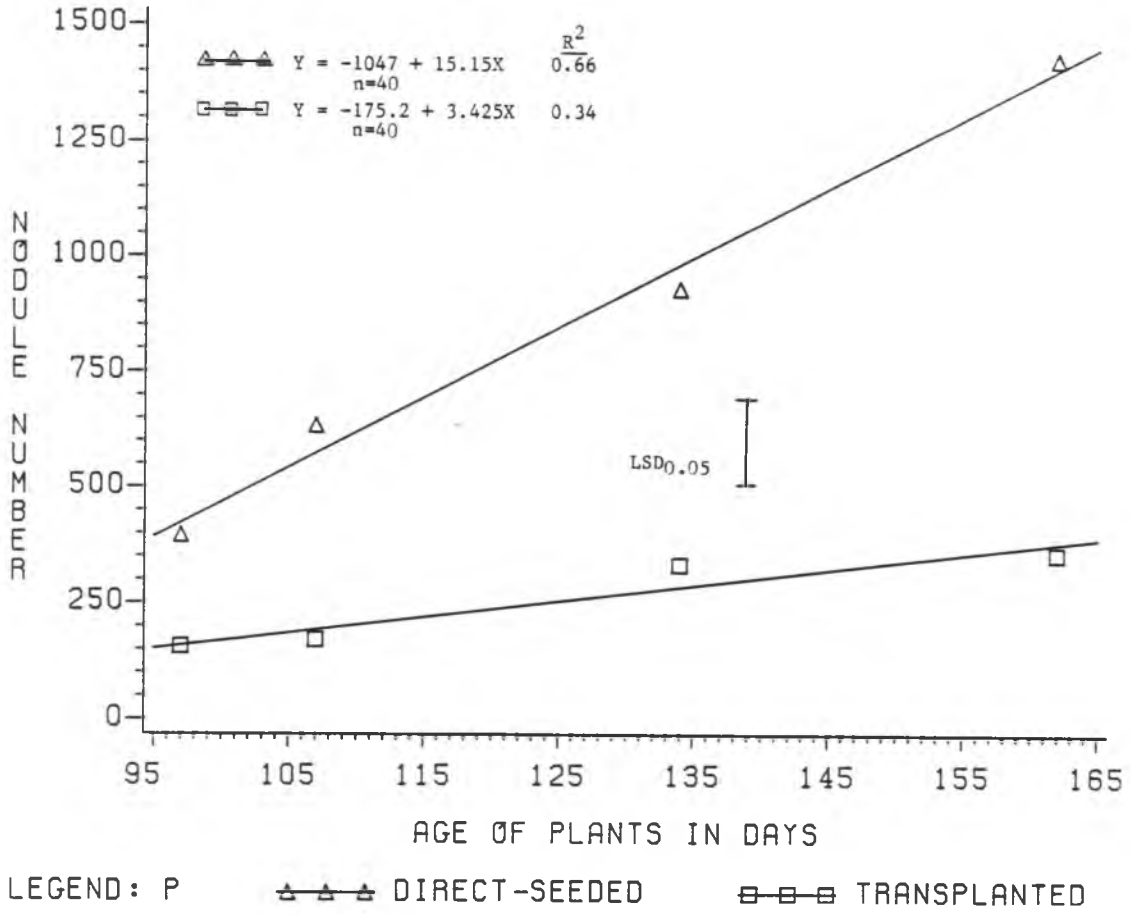

Fig. 16. Nodule number per plant at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

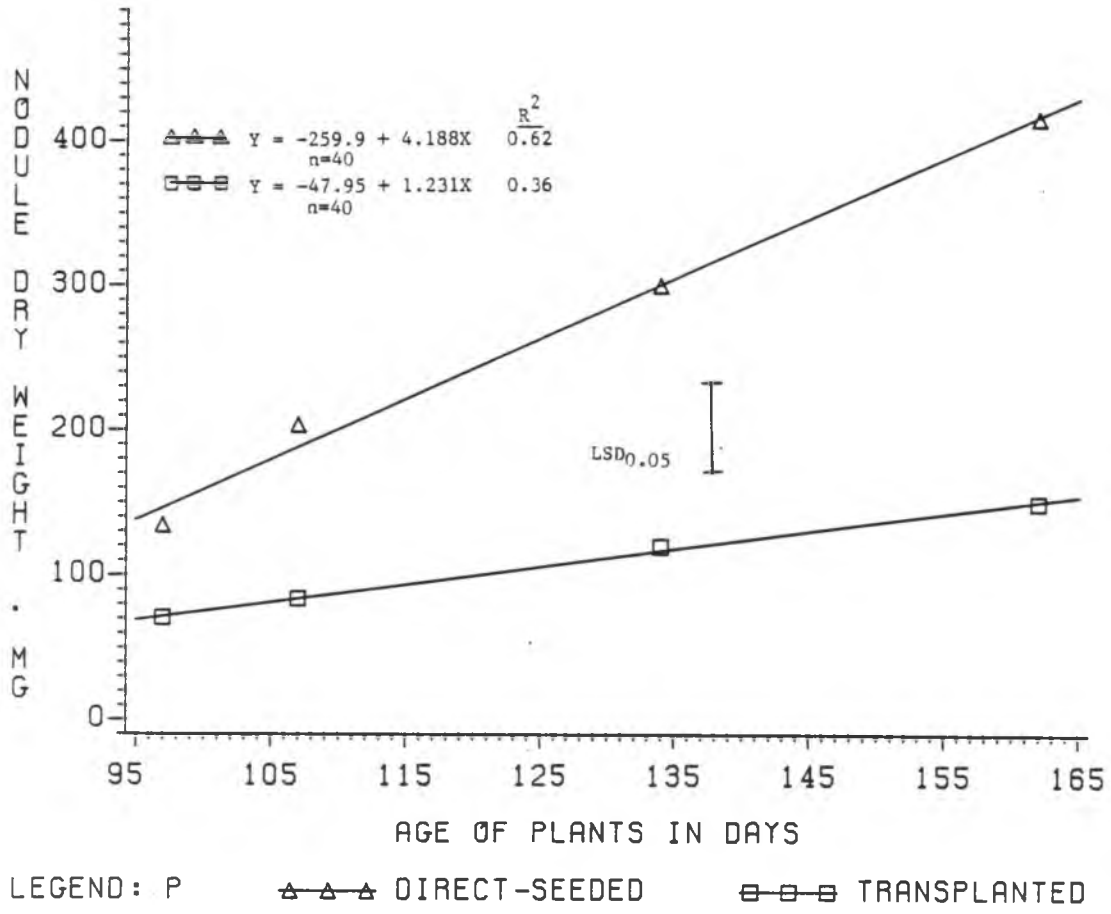

Fig. 17. Nodule dry weight per plant at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

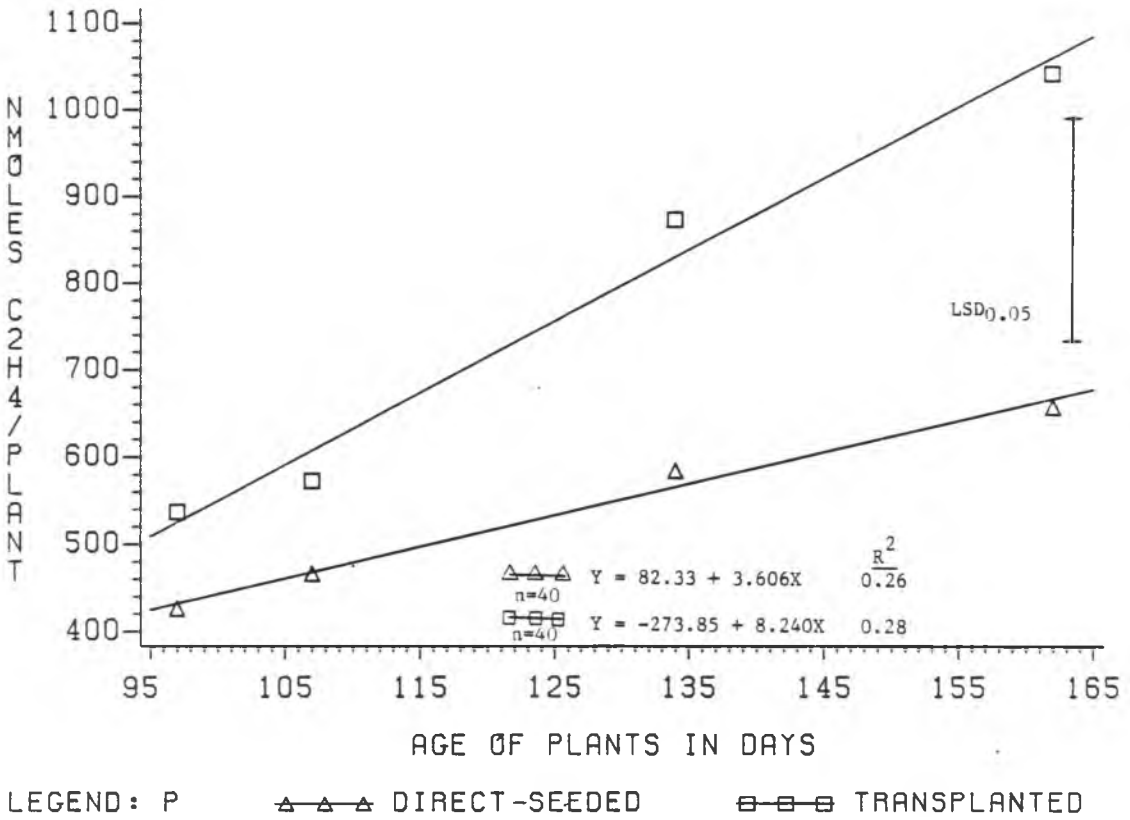

Fig. 18. Ethylene production per hour at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

transplanted and direct-seeded plants was not significantly different in the two younger age treatments.

For each age treatment, the transplanted leucaena exhibited significantly higher specific nodule activities than the direct-seeded plants (Fig. 19). In addition, the age x method of propagation interaction was significant. Specific nodule activity was negatively correlated with nodule number ($r = -0.71$), nodule dry weight ($r = -0.70$), and root dry weight ($r = -0.45$).

The shoot N concentrations for 97-day-old direct-seeded plants (25DS) were significantly higher than for 134-day-old direct-seeded plants [62DS (Fig. 20)]. Shoot N concentration of the transplanted leucaena did not differ significantly with age and remained constant at about 20 mg N/g shoot dry weight. Transplanted leucaena had significantly higher concentrations of shoot N at all ages than did the direct-seeded plants.

For any given age, the transplanted leucaena had significantly higher root N concentrations than their direct-seeded counterparts (Fig. 21). The root N concentrations for the transplanted leucaena averaged about 11 mg N/g shoot dry weight. Also, a significant age x method of propagation interaction was observed for this parameter.

Shoot N uptake did not vary significantly in the direct-seeded leucaena (Fig. 22). On the other hand, shoot N uptake in the transplanted leucaena increased with age. With the exception of the youngest plants, transplanted leucaena accumulated significantly more N than direct-seeded plants of the same age.

Both transplanted and direct-seeded leucaena showed increased root N uptake with age (Fig. 23). There was no significant effect of method of propagation on N uptake by the roots of plants in any age treatment.

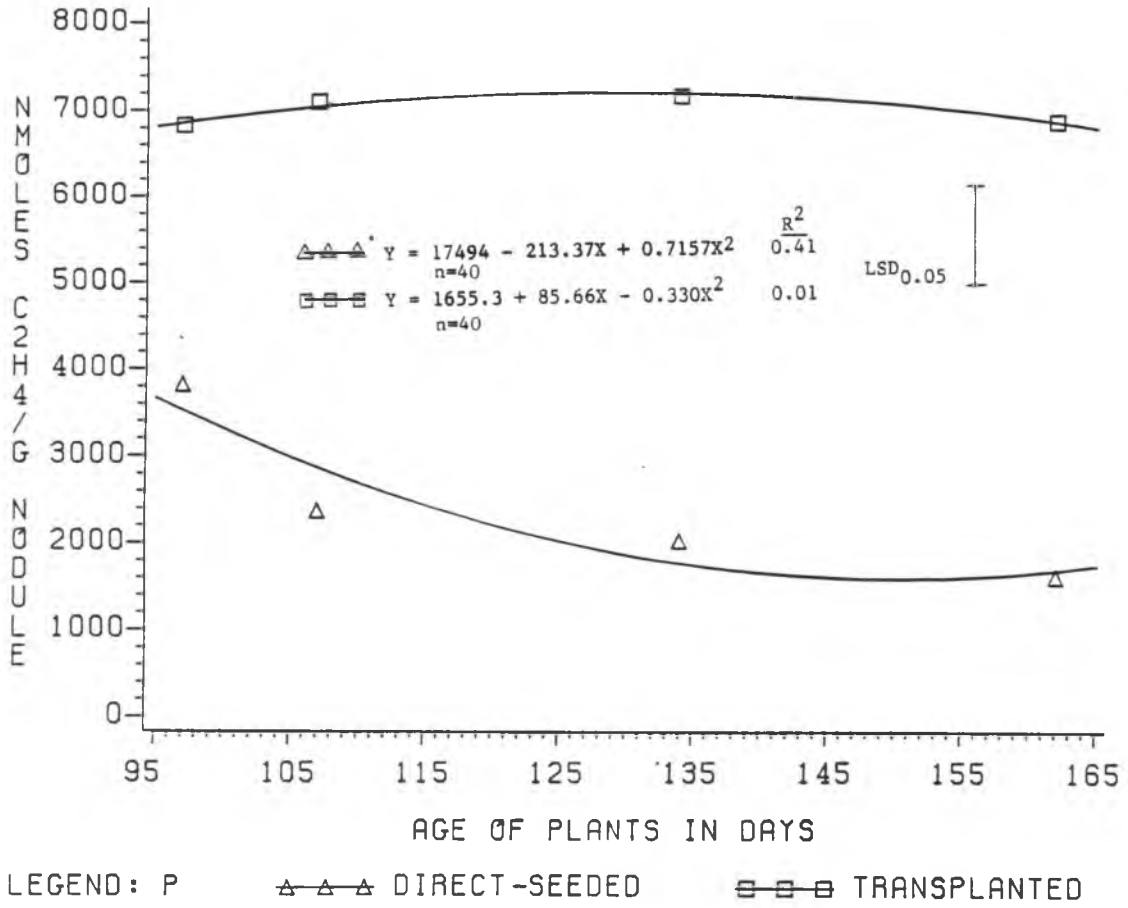

Fig. 19. Specific nodule activity per hour at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

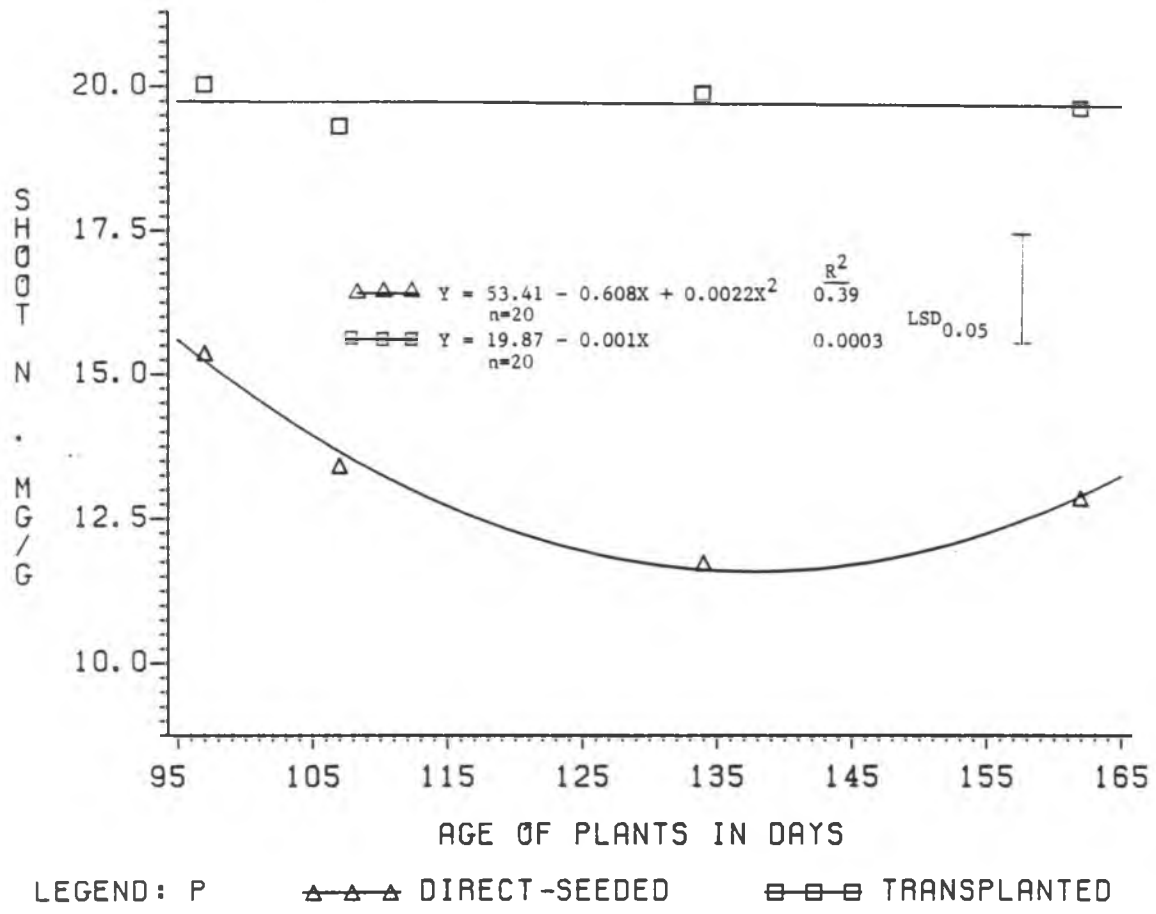

Fig. 20. Shoot N concentrations at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

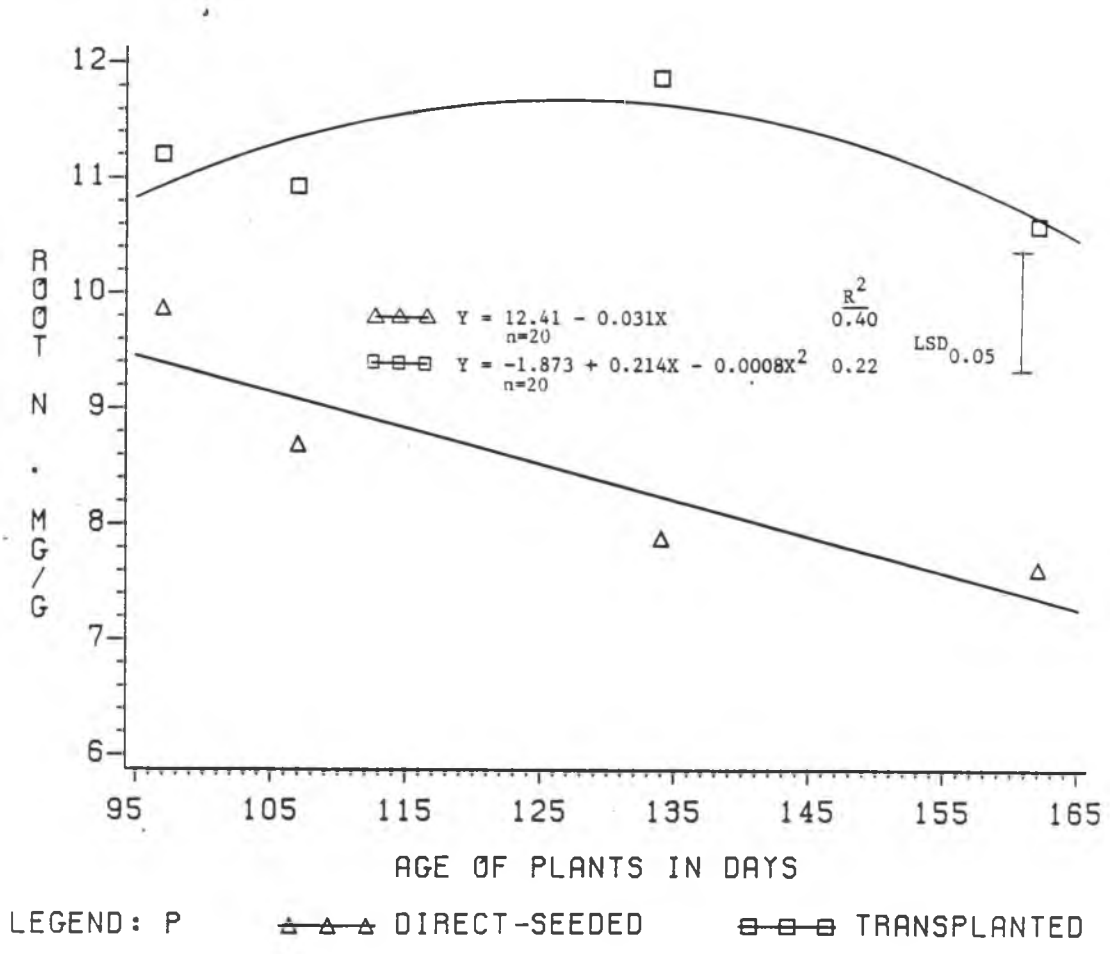

Fig. 21. Root N concentrations at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

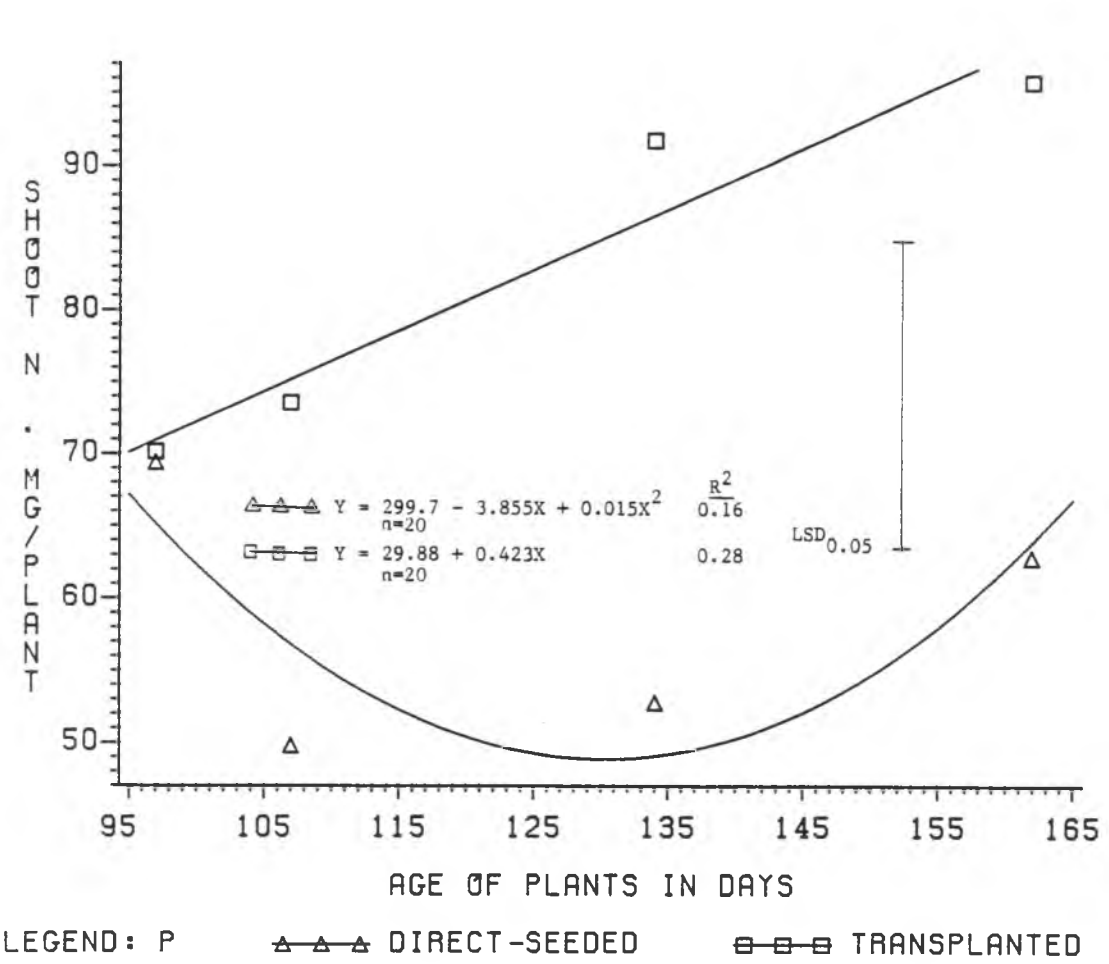

Fig. 22. Shoot N uptake at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

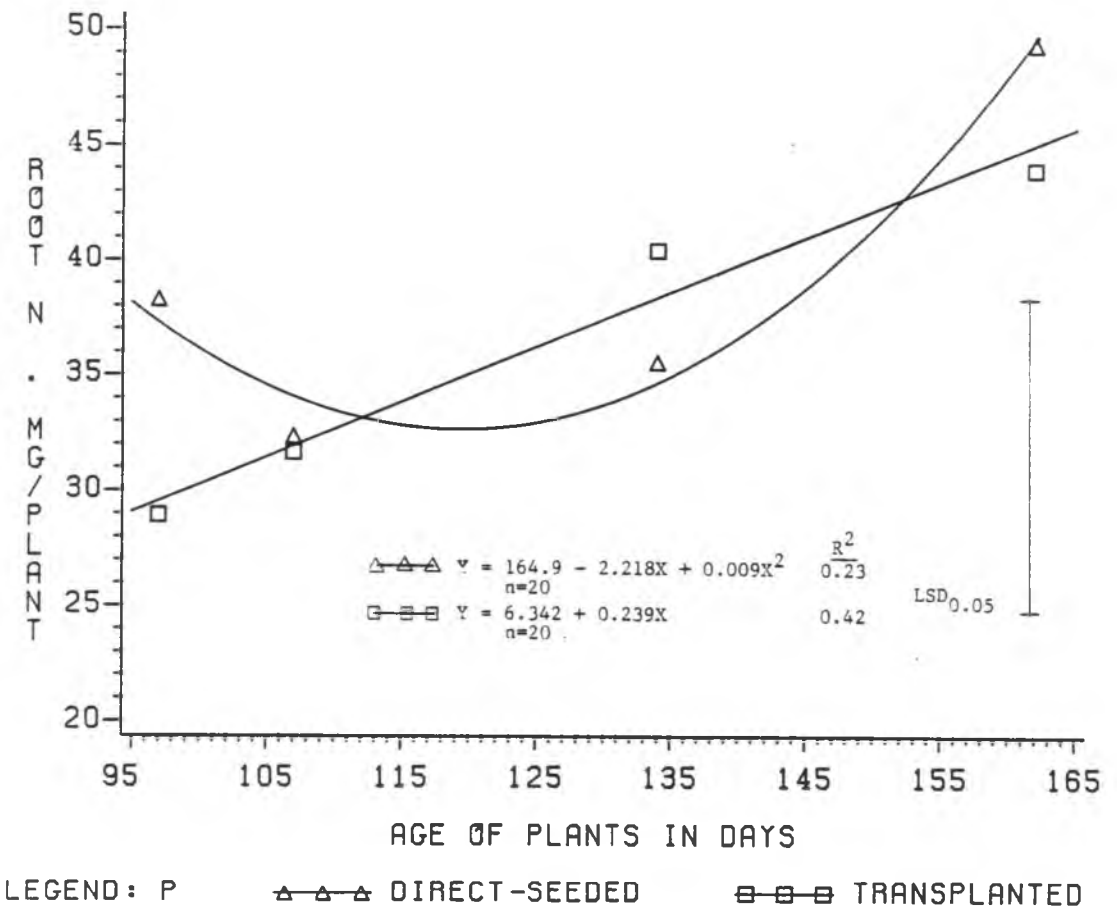

Fig. 23. Root N uptake at harvest for leucaena started in dibble tubes (TRANSPLANTED) and soil (DIRECT-SEEDED).

3. Nodule occupancy by *Rhizobium* sp. strain TAL582SR

Nodule occupancy by the antibiotic-resistant *Rhizobium* sp. strain TAL582SR was high in the transplants at the time of transplanting (Table 3). The lack of complete recovery of the inoculum strain from the 90-, 62-, and 35-day-old transplants at this time may have resulted from rhizobial contamination from air or water. The much lower nodule occupancy value recorded for the 25-day-old transplants at transplanting was probably not due to a more extensive contamination by other rhizobia but may have been due to the lack of a sizable number of TAL582SR bacteria in the small nodules. Also, the nodules from these young transplants were not easily manipulated, making it difficult to smear exudate from the crushed nodules onto the medium in the cluster plates. The low nodule occupancy values in the direct-seeded leucaena at transplanting and at harvest and in the transplanted leucaena at harvest suggest that infection and reinfection by strain TAL582SR in the soil was minimal. At the final harvest, nodules from all treatments were also crushed on cluster plates containing YMA amended with actidione alone and occupancy was nearly 100% (data not presented). This excellent growth on streptomycin sulfate- and demosan-free medium shows that most of the nodules possessed viable *Rhizobium* cells. It also eliminated the possibility that the bacteria might have been killed during the handling and plating of the nodules.

Table 3. Nodule occupancy by Rhizobium sp. strain TAL582SR.

Age (days)†	Method of propagation§	At transplanting	At harvest
		%	
90	T	80	0
62	T	97	0
35	T	88	4
25	T	46	1
90	DS	1	0
62	DS	1	3
35	DS	0	0
25	DS	0	2

† Age of plants started from direct-seeding or age of seedlings to be transplanted.

§ T = transplanted, DS = direct-seeded.

B. The Influence of Pre-transplanting Treatments on the Early Post-transplanting Performance of Leucaena and Its N_2-fixing Symbiont

This experiment was a follow-up of some preliminary studies (Appendices B, C, and D) and was conducted to evaluate the effects of pre-transplanting treatments on leucaena growth and its associated Rhizobium. The treatments included mycorrhizal inoculation (G. mosseae), 10 ppm starter N, 25 ppm starter N, G. mosseae inoculation plus 10 ppm starter N, G. mosseae inoculation plus 25 ppm starter N, and an uninoculated control. Leucaena seedlings were grown in dibble tubes and all were green and healthy when transplanted at 4 weeks of age. Midday temperatures in the dibble tube medium were recorded on two occasions prior to transplanting and reached 32°C.

At transplanting, the number of replicates used for each treatment was eight (one plant/replicate). The exceptions were Kjeldahl N (shoot and roots) and VAM infection determinations in which case four replicates were made into one sample due to a shortage of plant material needed for these determinations.

At transplanting, VAM infections were significantly higher in plants inoculated with G. mosseae and given 10 or 25 ppm starter N than in those plants given only G. mosseae inoculum (Table 4). Wherever present, infection of the root segments by G. mosseae was generally intense. Both external hyphae and arbuscles were prominent and a few vesicles were also observed. The root systems of leucaena seedlings treated with starter N alone and the control plants had no visible signs of VAM infection. The lack of infection in the uninoculated leucaena plants demonstrates that

Table 4. The influence of pre-transplanting treatments on several growth parameters of leucaena at transplanting.

Treatment†	PARAMETERS								
	Shoot dry weight	Root dry weight	Shoot/root ratio	Nodule dry weight/ plant	Nodule number/ plant	Shoot height	Shoot N	Root N	VAM infection
	-------- mg -------			mg		cm	------ mg/g -----		%
M	110.92 a§	53.42 a	2.08 a	5.84 a	7.88 ab	5.98 a	10.42 a	9.69 a	34.00 b
MN(10)	104.22 a	55.35 a	1.97 a	5.55 a	13.88 a	6.13 a	9.93 a	9.87 a	50.00 a
MN(25)	99.36 a	51.94 a	1.98 a	6.05 a	12.13 a	6.41 a	10.47 a	8.81 a	44.00 a
N(10)	108.04 a	53.86 a	2.03 a	4.15 a	8.25 ab	6.34 a	9.69 a	9.35 a	0 c
N(25)	112.19 a	55.94 a	2.11 a	3.44 a	5.00 b	5.91 a	10.39 a	9.56 a	0 c
Cm	96.35 a	50.46 a	1.95 a	4.94 a	10.63 ab	6.05 a	9.96 a	8.84 a	0 c
C.V.	16.9	24.3	26.4	61.0	62.6	12.8	6.0	6.3	12.1

† M = G. mosseae inoculation, MN(10) = G. mosseae plus 10 ppm N, MN(25) = G. mosseae plus 25 ppm N, N(10) = 10 ppm N, N(25) = 25 ppm N, Cm = uninoculated control.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

no mycorrhizal contaminants were introduced into the growth medium during or after planting.

Of the remaining parameters measured at transplanting, only nodule number was influenced by the treatments and the effect was small (Table 4). Plants inoculated with G. mosseae and given 10 or 25 ppm starter N had significantly more nodules than uninoculated plants given 25 ppm starter N alone. However, no consistent trend can be discerned from the results. Both nodule dry weight and nodule number were extremely variable (coefficients of variation greater than 60%). When the nodules were separated from the roots for drying, a few nodules from each seedling were split open to check the status of the leucaena-Rhizobium symbiosis. All of the nodule samples examined were effective N_2-fixers as suggested by their red interiors.

Four weeks after transplanting, the midday temperature of the potted soil in the greenhouse was 41°C. Plants not inoculated with G. mosseae were generally smaller and somewhat chlorotic while inoculated plants were larger and had normal coloring. Also, the uninoculated plants had a much greater percentage of senescing leaves. The number of healthy, expanded leaves per plant (11 plants/treatment) was significantly lower in the uninoculated leucaena plants (Table 5).

After the above greenhouse observations had been made, two plants per treatment were selected at random and the remaining parameters presented in Table 5 were measured. By the fourth week after transplanting, the plants not inoculated with G. mosseae were found to be mycorrhizal (Table 5). However, inoculation with G. mosseae in conjunction with both levels of starter N still resulted in significantly greater infection than the

Table 5. The influence of pre-transplanting treatments on several growth parameters of leucaena 4 weeks after transplanting.

Treatment†	PARAMETERS					
	Shoot dry weight	Nodule dry weight/ plant	Nodule number/ plant	Shoot height	VAM infection	Number of leaves/plant
	---------- mg ----------			cm	%	
M	381.6 a§	7.5 a	6.5 c	9.6 abc	39.0 ab	20.4 a
MN(10)	376.5 a	6.0 a	19.5 ab	9.7 ab	55.0 a	22.3 a
MN(25)	420.8 a	8.6 a	25.5 a	10.1 a	47.0 a	21.3 a
N(10)	197.4 b	2.8 a	9.0 abc	8.2 bcd	17.0 bc	12.7 b
N(25)	157.3 b	3.0 a	8.5 bc	7.9 d	10.0 c	11.2 b
Cm	134.1 b	2.9 a	11.0 abc	8.0 cd	18.0 bc	12.9 b
C.V.	19.3	44.8	42.4	6.8	29.9	21.8

† M = G. mosseae inoculation, MN(10) = G. mosseae plus 10 ppm N, MN(25) = G. mosseae plus 25 ppm N, N(10) = 10 ppm N, N(25) = 25 ppm N, Cm = uninoculated control.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

treatments not receiving G. mosseae inoculation. The infection level of plants inoculated with G. mosseae (but given no starter N) was over twice those of the uninoculated plants treated or untreated with 10 or 25 ppm starter N. However, the level of infection in this treatment (M) was significantly different only from the N(25) treatment. The intensity of VAM infection in the root segments did not appear to differ greatly among the treatments. However, vesicles were generally more numerous in the plants inoculated with G. mosseae. No spores were observed at this time. The VAM infection levels (as measured by counting the number of infected root segments) were positively correlated with shoot dry weight ($r = 0.88$) and shoot height ($r = 0.92$).

At 4 weeks after transplanting, a significantly larger accumulation of dry shoot material was recorded for leucaena plants receiving G. mosseae inoculation with or without starter N (Table 5). Plants inoculated with G. mosseae and given 25 ppm starter N produced the largest height increments and were significantly better than the uninoculated plants treated or untreated with starter N. However, no significant differences were recorded between the treatments receiving G. mosseae. Shoot height proved to be the least variable parameter measured in this study with a coefficient of variation of 6.8%.

No significant differences between treatments were noted for nodule dry weight (Table 5). However, leucaena plants inoculated with G. mosseae generally had greater nodule dry weights. With regard to nodule number, plants inoculated with G. mosseae and given 25 ppm starter N had significantly more nodules than plants from most other treatments (Table 5). Nodule dry weight at 4 weeks after transplanting was less than that

at transplanting for plants not inoculated with G. mosseae. Nodule dry weights for plants inoculated with G. mosseae with or without starter N were not much greater at 4 weeks after transplanting than at transplanting. Only a few of the nodules collected at this time appeared to be recently formed. Most were of moderate size (2 to 5 mm), indicating they were older and likely originated from the original TAL582SR inoculum. The N_2-fixing capacity of the root systems may not have been optimal at this time since a number of nodules examined at random from all treatments had green interiors.

Six weeks after transplanting, the number of leaves per plant and general plant health were assessed in the greenhouse (nine plants/treatment). In general, plants not inoculated with G. mosseae appeared greener and more vigorous than they were 2 weeks earlier. With regard to the number of leaves, the trend still favored plants inoculated with G. mosseae (Table 6). Leucaena plants inoculated with G. mosseae and given 10 or 25 ppm starter N had significantly more leaves than plants given only starter N. Plants inoculated with G. mosseae were not significantly different from each other in this regard. Leaf number of the uninoculated plants not treated with starter N also was not different from plants inoculated with G. mosseae.

The final harvest was made 8 weeks after transplanting. At this time, the midday soil temperature in the greenhouse was approximately 42°C. The number of replicates (one plant/replicate) used for treatment analyses was 7, with the exception of the VAM infection and nutrient concentration determinations in which case four and two replicates, respectively, were used.

Table 6. The influence of pre-transplanting treatments on the number of compound leaves of leucaena plants 6 weeks after transplanting.

Treatment†	Number of leaves/plant
M	30.44 ab§
MN(10)	37.67 a
MN(25)	33.67 a
N(10)	21.11 b
N(25)	19.22 b
Cm	26.22 ab
C.V.	40.8

† M = G. mosseae inoculation, MN(10) = G. mosseae plus 10 ppm N, MN(25) = G. mosseae plus 25 ppm N, N(10) = 10 ppm N, N(25) = 25 ppm N, Cm = uninoculated control.

§ Means within column followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

At 8 weeks after transplanting, plants in all treatments were uniformly mycorrhizal (Table 7). Vesicles were quite common in most infected root segments. A number of spores were observed in plants which had been inoculated with G. mosseae. In the uninoculated plants, the presence of spores was a rarity and was observed only in a few plants which received 10 ppm starter N.

Shoot dry weights of inoculated plants with or without starter N were greater than those of plants given only starter N but were not significantly different from uninoculated plants not treated with starter N (Table 7). A somewhat similar trend was seen for root dry weights (Table 7). Shoot heights of plants inoculated with G. mosseae with or without starter N were greater than those of the uninoculated control and plants given 25 ppm starter N only (Table 7). The heights of inoculated plants were not significantly different from that of plants given only 10 ppm starter N. The number of leaves on plants from the inoculated treatments did not differ significantly from those of the uninoculated control but equalled or exceeded plants given only starter N (Table 7).

The shoot/root ratios at harvest tended to be greater for uninoculated plants than for inoculated ones (Table 7). However, few differences were statistically significant.

Shoot and root N concentrations were not significantly different between treatments at 8 weeks after transplanting (Table 7). Concentrations of other elements in the shoots and roots were also determined and are presented in Tables 8 and 9. The differences between treatments were generally small, but some differences were significant. The concentrations of shoot P, K, and S were significantly lower in plants inoculated with G. mosseae and given 25 ppm N (Table 8). The addition of both

Table 7. The influence of pre-transplanting treatments on several growth parameters of leucaena 8 weeks after transplanting.

Treatment†	PARAMETERS							
	Shoot dry weight	Root dry weight	Shoot/root ratio	Shoot height	Shoot N	Root N	VAM infection	Number of leaves/plant
	-------- g --------			cm	----- mg/g -----		%	
M	1.12 a§	0.82 ab	1.33 b	18.76 a	33.40 a	21.30 a	68.00 a	44.29 ab
MN(10)	1.25 a	0.96 a	1.37 b	18.39 a	34.20 a	19.85 a	84.00 a	52.00 a
MN(25)	1.14 a	0.83 ab	1.41 ab	17.71 a	32.85 a	21.60 a	69.50 a	46.29 ab
N(10)	0.55 b	0.33 c	1.63 ab	16.01 ab	41.85 a	24.30 a	66.00 a	29.29 c
N(25)	0.54 b	0.32 c	1.76 a	13.74 b	40.20 a	23.15 a	67.50 a	33.86 bc
Cm	0.84 ab	0.57 bc	1.49 ab	13.19 b	36.35 a	22.90 a	64.50 a	41.29 abc
C.V.	41.6	44.5	21.3	20.4	11.3	16.1	29.7	27.1

† M = G. mosseae inoculation, MN(10) = G. mosseae plus 10 ppm N, MN(25) = G. mosseae plus 25 ppm N, N(10) = 10 ppm N, N(25) = 25 ppm N, Cm = uninoculated control.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

Table 8. The influence of pre-transplanting treatments on the nutrient content of leucaena shoots 8 weeks after transplanting.

Treatment†	PARAMETERS									
	P	K	Ca	Mg	S	Si	Mn	Fe	Cu	Zn
	%						ppm			
M	0.19 a§	2.04 a	0.85 a	0.26 ab	0.21 a	0.04 a	57.5 ab	256.5 b	21.5 a	28.0 a
MN(10)	0.18 a	1.94 a	0.86 a	0.27 ab	0.21 a	0.04 a	63.5 a	291.0 ab	19.5 ab	19.0 bc
MN(25)	0.14 b	1.64 b	0.86 a	0.28 a	0.19 b	0.06 a	64.5 a	348.0 ab	16.0 b	12.0 c
N(10)	0.18 a	1.97 a	0.85 a	0.27 ab	0.20 a	0.05 a	60.5 ab	332.5 ab	22.0 a	27.0 ab
N(25)	0.19 a	2.02 a	0.77 a	0.24 b	0.21 a	0.04 a	51.5 b	251.0 b	20.5 ab	24.5 ab
Cm	0.20 a	2.23 a	0.80 a	0.26 ab	0.21 a	0.05 a	66.0 a	393.0 a	20.0 ab	22.5 ab
C.V.	5.5	5.8	6.4	5.3	2.6	20.6	7.2	14.2	8.8	14.2

† M = G. mosseae inoculation, MN(10) = G. mosseae plus 10 ppm N, MN(25) = G. mosseae plus 25 ppm N, N(10) = 10 ppm N, N(25) = 25 ppm N, Cm = uninoculated control.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

Table 9. The influence of pre-transplanting treatments on the nutrient content of leucaena roots 8 weeks after transplanting.

Treatment†	PARAMETERS									
	P	K	Ca	Mg	S	Si	Mn	Fe	Cu	Zn
	%						ppm			
M	0.13 a§	1.85 ab	0.26 b	0.33 a	0.16 b	0.34 b	118.0 c	865.5 b	20.0 a	20.0 bc
MN(10)	0.16 a	2.34 a	0.34 a	0.50 a	0.24 a	1.06 a	383.5 a	4032.0 a	14.0 a	41.0 a
MN(25)	0.15 a	2.15 ab	0.28 ab	0.40 a	0.23 a	0.77 a	272.0 b	2779.5 a	17.5 a	37.0 ab
N(10)	0.14 a	1.97 ab	0.32 a	0.41 a	0.23 a	0.88 a	293.5 b	3094.0 a	12.0 a	32.5 ab
N(25)	0.13 a	1.94 ab	0.33 a	0.48 a	0.21 a	0.97 a	309.5 ab	3616.5 a	4.0 a	27.5 abc
Cm	0.13 a	1.70 b	0.24 b	0.32 a	0.15 b	0.38 b	128.5 c	934.5 b	16.0 a	11.5 c
C.V.	9.6	11.2	7.1	18.0	9.2	16.7	11.9	23.1	53.0	20.1

† M = G. mosseae inoculation, MN(10) = G. mosseae plus 10 ppm N, MN(25) = G. mosseae plus 25 ppm N, N(10) = 10 ppm N, N(25) = 25 ppm N, Cm = uninoculated control.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

levels of starter N in the presence or absence of G. mosseae significantly increased root concentrations of S, Si, Mn, and Fe (Table 9).

When the roots of the leucaena plants were being washed at this final harvest, most root systems had no nodules. The few plants with nodules appeared to be scattered randomly among the treatments and were generally larger than plants without nodules in the same treatments (data not presented).

V. DISCUSSION

A. The Influence of Propagation Methods and Age of Transplanting on the Performance of Leucaena and Its N_2-fixing Symbiont

In this experiment, the effects of direct-seeding and transplanting on leucaena growth and N_2-fixation were compared for plants in four age groups. Also, the performance among the transplants was evaluated to allow the selection of the "best" transplanting age.

The total growth of the leucaena plant was partitioned into several measurable parameters whose values differed with the age of the plant and the environment in which it was raised. One influential variable that exerted control over leucaena growth prior to transplanting was the container size, i.e., the 0.05 liter dibble tube and 2.6 liter pot. The fifty-fold difference in container size accounted for some significant differences in root growth with increasing age. Compared to the leucaena directly-seeded into pots, the root dry weight of the transplants was significantly smaller by 62 and 90 days of age. This reduction in root growth in the transplants with age was reflected in significantly lower shoot dry weight, shoot height, nodule number, nodule dry weight, and total shoot and root uptake of N relative to the direct-seeded plants. Placing limits on root growth reduced the effective surface area for taking up nutrients and water from the soil, thereby inhibiting other aspects of leucaena growth. There were differences in the two growth media used for growing the leucaena plants; however, it is likely that their effect in dictating differences in plant growth would have been comparatively smaller than that of container size. Limitation of seedling

growth with small containers has been reported for a number of plant species that are commonly transplanted (Knavel, 1964; Wang and Kratky, 1976; Wang et al., 1979; Kratky et al., 1982).

The responses of a few parameters measured at transplanting were apparently not directly related to the method of propagation. For example, the significantly higher shoot N concentration for the 25-, 35-, and 62-day-old direct-seeded plants may have been due to a better N nutrition provided by the Waialua soil (as opposed to the dibble tube medium) prior to active N_2-fixation. At transplanting, the shoot/root ratio decreased with age but differences were not significant between plants grown in dibble tubes and pots. This observation suggests that the shoot/root ratio for leucaena at a given age remains fairly constant despite the overall differences in plant size that may be dictated by differences in the size of growth containers. The decrease in the shoot/root ratio with age differs from the pattern of ratios reported for plants such as corn, wheat, soybeans and peas which show an increase in the shoot/root ratio with age (Aung, 1971; Boote, 1976). Shoot/root ratios have also been reported to vary with plant species as well as with chronological age, state of morphological development, and the growth environment (Aung, 1971). Since leucaena is a taproot-forming woody perennial and the decrease in shoot/root ratio with age as seen here may be a normal characteristic of this plant during this early phase of growth.

Corresponding harvest measurements were made for parameters that were evaluated at the time of transplanting. A concern at this harvest was the extent of root exploration by the potted leucaena plants. In pot experiments of long duration, plants may sometime become "potbound"

and treatment effects may be masked (Linderman and Hendrix, 1982). The root growth in both direct-seeded and transplanted leucaena was roughly linear at harvest and the lines did not converge over the ages studied. These results suggest that the older direct-seeded leucaena plants may not have been strongly potbound, at least not to any greater degree than the transplanted leucaena. A certain amount of "potbounding" will always occur in greenhouse-grown leucaena unless the plants are grown in containers large enough so that the edges are not reached by the roots during the experiment.

A comparison of the root dry weights at transplanting and harvest for the transplanted leucaena showed large increases in size after being placed in the larger pots containing the Waialua soil. However, the root dry weights of leucaena plants transplanted at 62 and 90 days of age were still smaller at harvest than those of direct-seeded plants of the same age. Reflecting the trend for root dry weights, nodule numbers and nodule dry weights for the transplants were significantly smaller than for correspondingly-aged direct-seeded plants.

In contrast to the trends reported at harvest for the above parameters, shoot dry weight was not significantly affected by method of propagation at this time. Moreover, transplanted leucaena were significantly better at harvest with regard to shoot heights and shoot/root ratios (35T, 62T, 90T transplants), shoot and root N concentrations (all ages), and total N uptake by the shoots (35T, 62T, and 90T transplants). The better performance of the transplants in these parameters may have been due to their significantly higher acetylene reduction (62T, 90T transplants) and specific nodule (all ages) activities. The higher N_2-fixing status in these transplants was likely responsible for better N nutrition which was

in turn expressed in the shoot growth and N concentrations of the plant tissues. The specific nodule activity, an indicator of the efficiency with which N_2 is fixed in the nodules, may have been higher in the transplants because they had nodules that were somewhat larger than the direct-seeded plants. It has been reported that large nodules are generally more efficient N_2-fixers than smaller ones (Alexander, 1977a). The specific nodule activity was negatively correlated with nodule number ($r = -0.71$) and nodule dry weight ($r = -0.70$), suggesting that a large proportion of the more numerous nodules in the direct-seeded plants were inefficient in their N_2-fixing activities. Another cause for the higher N_2-fixing activity in the transplants may have been due to the fact that most nodules formed on these plants occurred after transplanting while direct-seeded plants (especially the two oldest age groups) had many nodules prior to transplanting. It is possible that the older direct-seeded plants could have had a correspondingly greater number of senescing nodules at harvest than the younger direct-seeded and all transplanted leucaena plants (assuming a minimum nodule life span of 2 to 3 months for greenhouse-grown leucaena). This would help account for the significantly lower acetylene reduction values observed in the two older ages of direct-seeded plants when compared to transplanted leucaena of the same age. This suggestion, however, in no way implies that no new nodules were being formed on the roots of these direct-seeded plants at the time of harvest. In the field, differences that are attributed to nodule health might disappear after a time due to the establishment of an equilibrium between newly forming and senescing nodules in both transplanted and direct-seeded leucaena plants.

The N_2-fixing activity of leucaena observed in this study was low when compared to that observed in many annual legumes. The specific nodule activity of greenhouse-grown soybean has been reported to have a range of 130 to 250 umoles ethylene/g nodule dry weight hour (Graham and Halliday, 1977). Reported activities for N_2-fixing trees are somewhat lower. The specific nodule activity recorded in the present study is comparable to that reported for the Mexican plantings of Inga jinicuil (Roskoski, 1981) and slightly lower than that reported for 4 year old L. leucocephala grown in Tanzania (Hogberg and Kvarnstrom, 1982).

Nodule occupancy tests revealed that Rhizobium sp. strain TAL582SR effectively nodulated leucaena seedlings in the sterile peat-vermiculite medium in the dibble tubes but that nodulation by this strain was minimal in the unsterile Waialua clay soil. Therefore, most of the nodulation of the leucaena roots in the soil environment was due to indigenous rhizobia. This finding is in conflict with that of Jones (1977) who observed good infection of field-grown leucaena in Australia by the introduced strain CB81 (TAL582). The results of the present study further suggest that the method of propagation and age of the leucaena seedlings at transplanting had no bearing on nodule occupancy by Rhizobium sp. strain TAL582SR.

A large number of Rhizobium cells (10^8 cells/seed) were used for inoculation in this study. This shows that inoculation with a large number of Rhizobium cells at planting does not necessarily ensure the prevalence of inoculum strains against well-established indigenous rhizobial populations. These results are in agreement with those of Kuykendall and Weber (1978) who found that inoculation of soybean with 3 x 10^8 cells/seed of a streptomycin-labelled strain of R. japonicum

resulted in only about a 5% recovery of the labelled strain from the nodules.

It may be that strain TAL582SR survived poorly in the Waialua soil relative to the indigenous rhizobia. Several researchers have reported differences in survival of strains of R. trifolii and R. japonicum in several unsterile soils (Johnson et al., 1965; Gibson et al., 1976; Ayanaba and Wong, 1982; Guar and Lowther, 1982). A number of soil factors such as acidity, temperature, nutrients, and microbial antagonism can differentially influence the survival of Rhizobium strains (Wilkins, 1967; Edwards, 1977; Pena-Cabriales and Alexander, 1983).

Another possible reason for the low nodule occupancy levels in the soil may be that strain TAL582SR competed poorly with the indigenous rhizobia for nodulation sites on the roots. Investigators have observed in the greenhouse and field that infective R. japonicum and R. trifolii strains may be quite similar in terms of effectiveness while differing in their competitiveness for nodule sites (Amarger, 1981b; Guar and Lowther, 1982).

Even though TAL582SR may not persist as the major Rhizobium symbiont in the Waialua soil over the long term, the nodulation of leucaena by this strain in the sterile dibble tube medium may benefit the growth of the seedlings before and shortly after transplanting. Yet, a continuing presence of the N_2-fixing symbiosis will depend on post-transplanting reinfection by effective soil rhizobia if the introduced Rhizobium strain is a poor competitor or survives poorly in the rhizosphere. The indigenous rhizobia in the Waialua soil were shown to be effective in their association with leucaena. However, it remains to be seen if these rhizobia form the most beneficial N_2-fixing symbiosis possible. Recently,

interest in the Rhizobium sp. strain TAL1145 has been building. This strain shows promise for selectively nodulating leucaena in the presence of indigenous rhizobial populations (B. Bohlool, personal communication).

Within the framework of this study, leucaena was not adversely affected by transplanting. Based on one of the most important agronomic features of leucaena, i.e., shoot growth, there was no significant difference between the two methods of propagation at harvest. Furthermore, significantly lower harvest values for root growth, nodule number, and nodule dry weight in the transplanted leucaena were countered by higher specific nodule activity, resulting in higher shoot and root N concentrations. These results suggest that under the conditions prevailing in the greenhouse, either method could be used to ensure survival and good growth. An important consideration is the economic feasibility of one method of leucaena establishment over the other. Direct-seeding is often cheaper because one eliminates the labor and maintenance costs that are associated with the production of a transplantable seedling and placement in the field. In many situations, however, transplanting may be the only way to establish a stand of leucaena. It was noted in this study that between 10 and 20 seeds were needed per pot of Waialua soil to establish only a few direct-seeded plants. This was due to high seedling mortality caused by the fungi Rhizoctonia sp. and Phytopthora sp. Therefore, in soils where pathogenic fungi are present, a large number of seeds may have to be broadcast to ensure the survival of the direct-seeded leucaena. In the field, competition from weeds can be a further impediment in the establishment of the leucaena plant (Vietmeyer and Cottom, 1979). Brewbaker and Hutton (1979) reported that the establishment of direct-seeded leucaena is slow compared to that of transplanted

leucaena while survival rates of nearly 100% have been observed for leucaena transplanted from dibble tubes. This suggests a survival advantage in transplanting seedlings which can become established before the weeds.

With regard to comparisons among the transplants, it was noted at the time of transplanting that the shoot and root growth of 25- and 35-day-old transplants were not yet significantly limited by dibble tube size. However, the roots of these seedlings were not sufficiently developed to allow the removal of the growth medium and roots as a single unit. This increased the chances of mechanically injuring the root during the transplanting process. On the other hand, 62- and 90-day-old transplants possessed more extensive root systems which were more easily removed from the dibble tubes with the growth medium intact. At the time of harvest, leucaena plants transplanted at 25 and 35 days of age were still statistically inferior to those transplanted at 62 and 90 days of age with regard to a number of growth parameters. At this time, the leucaena transplanted at 62 and 90 days of age were not significantly different from each other (except in root N concentration). Since the performance of the 90-day-old transplants was not significantly better than that of the 62-day-old ones, there appears to be no advantage in keeping seedlings in dibble tubes for an additional month. The results of this study, therefore, suggest that 62 days is the best age (under the conditions of this experiment) at which to transplant leucaena from dibble tubes.

B. The Influence of Pre-transplanting Treatments on the Early Post-transplanting Performance of Leucaena and Its N_2-fixing Symbiont

The effects of pre-transplanting treatments on the early post-transplanting growth of leucaena and its associated Rhizobium were evaluated. The treatments included a mycorrhizal inoculation with G. mosseae, a starter N application (10 or 25 ppm N), combinations of these treatments, and an uninoculated control.

In the present study, the most important component involved in the improvement of post-transplanting growth was an initial inoculation with G. mosseae. The addition of 10 or 25 ppm starter N alone gave fewer significant growth advantages than inoculation with G. mosseae. When the two levels of starter N were combined with G. mosseae inoculation, responses were generally similar to G. mosseae inoculation without the starter N.

The beneficial effects of G. mosseae on plant growth were not significant at transplanting but after 4 weeks of growth in the Waialua soil, the contrast between inoculated and uninoculated leucaena plants was striking. Inoculation with G. mosseae reduced leaf losses and prevented the development of chlorosis. This was reflected in the significantly higher numbers of compound leaves and shoot dry weights. These findings are consistent with those of Sinclair and Marx (1982) who reported that VAM fungi may influence dry weight accumulation in several plant species within 2 months after planting.

Leucaena plants inoculated or uninoculated with G. mosseae achieved similar levels of mycorrhizal infection 8 weeks after transplanting into the Waialua soil and the growth lead held by the inoculated plants was

decreased. However, the growth advantage resulting from the initial inoculation with G. mosseae was still apparent for a number of parameters which included shoot and root dry weights, shoot height, and compound leaf number. The diminishing gap between the performance of inoculated and uninoculated plants suggests that G. mosseae was not qualitatively more effective than the indigenous VAM fungi. Therefore, the real benefit of the G. mosseae inoculation at planting was the development of an earlier infection. This observation is in agreement with that of Islam (1977) who reported that mycorrhizal cowpea transplants which had an initial advantage over seed sown into the field at the time of transplanting lost this advantage after 7 weeks.

By the time of transplanting, the combination of G. mosseae and starter N (10 or 25 ppm N) had induced more infections in the dibble tube seedlings than the addition of G. mosseae alone. This addition of 10 or 25 ppm starter N in the form of NH_4NO_3 may have raised the amount of immediately available N in the peat-vermiculite medium to a level which provided for increased infection of the roots. In spite of these differences in infection level for plants receiving G. mosseae and plants receiving G. mosseae plus starter N, differences in growth parameters were mostly nonsignificant in these plants at transplanting and subsequent observations.

Nitrogen concentrations measured at transplanting and at final harvest were not significantly different. However, between the time of transplanting and final harvest, N concentrations more than doubled in both shoots and roots. This finding is in contrast to the trend of decreasing N concentrations with age reported in the previous section where the effects of method of propagation and age of transplanting were

examined. The tissue concentrations of elements other than N were measured only at the time of the final harvest because of the large amount of dry matter required for X-ray quantometer analyses. Only one trend was noted and this was with regard to increased S, Si, Mn, and Fe concentrations in roots with addition of both levels of starter N in the presence or absence of G. mosseae inoculation. This trend suggests a positive role for the starter N applications exclusive of the G. mosseae inoculation. It also shows that increases in elemental concentrations can occur independently of some of the growth responses. De Vries (1980) remarked that rather wide concentrations of several elements can occur in many possible combinations without changing a plant's behavior. The nutrient concentrations reported in the present study for leucaena are roughly comparable to those reported by other researchers (Hutton, 1982; Van den Beldt, 1982). Caution is necessary when these values are applied elsewhere because the age of the plant (de Vries, 1980) and the nutrient content of the soil (Small and Ohlrogge, 1980) can affect the concentrations of nutrients in the plant.

An examination of the root systems 4 weeks after transplanting showed that few new nodules were forming. Many of the nodules that were sliced open were green inside, perhaps signifying the beginning of nodule breakdown. By the final harvest (8 weeks after transplanting), most leucaena plants were devoid of nodules. This deterioration and loss of nodules eliminated the possibility of assessing the tripartite association between leucaena, Rhizobium, and G. mosseae in this experiment. Since the soil that was used throughout all experiments was from the same source, major differences in soil composition can be ruled out as a cause of poor nodulation. A likely cause of the poor nodulation observed here was high

greenhouse temperatures. This experiment was initiated in May and was carried through the hottest part of summer. The greenhouse in which the leucaena plants were grown was inadequately ventilated and soil temperatures reaching 42°C at midday were recorded. Prior to transplanting, dibble tube temperatures did not exceed 32°C.

Extra plants from the previous part of this thesis study (method of propagation, age of transplanting treatments) had been retained and maintained in the greenhouse. These plants had been transplanted in January when it was cooler. They were well nodulated before the approach of summer and still had many effective nodules with red interiors when sampled in August. This finding suggests that poor nodulation in the present experiment was due to a temperature-related elimination of the soil rhizobia before they could adequately infect the root systems of the transplanted leucaena (on the basis of the earlier study, it is assumed that continued nodulation in the soil environment by TAL582SR was minimal). Although the rhizobia in the soil are generally more susceptible to temperature stress than the legume, high temperatures can act on both members to inhibit nodulation (Wilkins, 1967; Danso and Alexander, 1974; Friere, 1977; Osa-ofiana and Alexander, 1979). After nodules are initiated, however, the effects of high temperatures on nodule development and N_2-fixation are often less pronounced (Danso, 1977; Friere, 1977). Date (1977b) observed that soil temperatures ranging between 31°C and 43°C can restrict and reduce the number of cowpea, pea, and clover rhizobia in soil. As a consequence of the reduced survival of soil rhizobia, several leguminous species show severely reduced nodulation and depression of N_2-fixation where soil temperatures frequently exceed 35°C

to 40°C for several hours daily (Graham and Halliday, 1977; Kang et al., 1977; Munevar and Wollum, 1982).

In addition to the effects on nodulation, the stresses imposed on the plants in this study may account to some extent for the differences in health between the plants inoculated with G. mosseae and those that were not inoculated. As discussed earlier, the plants inoculated with G. mosseae appeared mostly green and healthy after transplanting while the uninoculated plants experienced chlorosis and leaf loss. In the previous study where the method of propagation and age of transplanting effects were examined, the transplanted seedlings had not been inoculated with G. mosseae but showed few signs of chlorosis and leaf loss, or other symptoms of ill health in the month following transplanting (January). Therefore, inoculation with G. mosseae must have helped plants to tolerate the greenhouse stresses. Barrows and Roncadori (1977) observed that a mycorrhizal association with the fungus Gigaspora margarita reduced transplant shock in poinsettia (Euphorbia pulcherrima) grown under high temperatures.

In conclusion, this study has shown that inoculation with the mycorrhizal fungus G. mosseae at the time of planting can improve the early post-transplanting growth of leucaena, even in the presence of stress conditions. For the most part, the two levels of starter N when used alone were apparently not sufficient to generate significant growth responses. Also, plants given both G. mosseae inoculation and starter N (10 or 25 ppm) generally performed similarly to plants given just the G. mosseae inoculation. This implies that the benefits imparted by the starter N in the combination treatments were small when compared to those generated by the G. mosseae infection. Nonetheless, the positive results

shown with the G. mosseae/starter N combination (e.g., increased mycorrhizal infection at transplanting) warrant further use of either level of starter N in conjunction with mycorrhizal inoculation. The advantages of G. mosseae inoculation were diminished at 8 weeks after transplanting, a time when uninoculated plants had exhibited good infection from indigenous VAM fungi. However, the initial growth lead imparted by G. mosseae could be an important factor in ensuring the survival of leucaena seedlings in an inclement environment. I suggest that additional work be conducted in a more carefully controlled greenhouse situation, especially if a more complete analysis of the leucaena-Rhizobium-G. mosseae symbiosis is to be accomplished. Mycorrhizal leucaena should be transplanted into the field preferably in a marginal environment where the survival benefits of the G. mosseae infection could be better tested.

VI. SUMMARY

The main objectives of this investigation entailed examining the effects of method of propagation, age of transplanting, and pre-transplanting treatments on the early growth and N_2-fixation potential of greenhouse-grown L. leucocephala (Lam.) de Wit. Overall, transplanting did not compromise either plant growth or the N_2-fixing symbiosis. Based on the most important agronomic features of leucaena, i.e., shoot growth, there was no significant difference between the two methods of propagation (all ages) at harvest. Furthermore, significantly lower harvest values for root growth (62T, 90T transplants), nodule number and nodule dry weight (all ages) in the transplanted leucaena were countered by higher acetylene reduction (62T, 90T transplants) and specific nodule activities (all ages), and higher shoot and root N concentrations (all ages). The higher levels of N2-fixation in the transplanted leucaena may have been due to larger nodule size and/or smaller number of senescing nodules in these plants. These results suggest that under the conditions prevailing in the greenhouse, either method could be used to ensure good growth. However, competition from weeds or infection by fungal pathogens may reduce survival of direct-seeded plants and, therefore, make transplanting the only practical way to establish leucaena in the field.

Of the 4 ages tested, 62 days appeared to be the best age at which to transplant leucaena. This conclusion was based on an assessment of plant performance at both the time of transplanting and harvest. Although the effect of age was not significant at transplanting, the 62- and 90-day-old seedlings had more extensive root systems and were more easily removed from the dibble tubes than the younger seedlings. By the time of

harvest, the leucaena seedlings transplanted at 25 and 35 days of age were still statistically inferior to those transplanted at 62 and 90 days of age with regard to shoot and root dry weight, shoot height, and acetylene reduction activity despite container restrictions imposed on plants from the latter two treatments prior to transplanting. Leucaena transplanted at 62 and 90 days of age did not differ significantly from each other for most parameters measured. Therefore, it seems uneconomical to grow seedlings for up to 90 days in the dibble tubes when 62-day-old seedlings give similar results within 72 days after transplanting. Also, the ease of seedling handling during transplanting and higher early yields may compensate for increased costs associated with growing seedlings to 62 days of age instead of 25 or 35 days.

The method of propagation and age of the leucaena seedlings at transplanting probably had no bearing on nodule occupancy by the Rhizobium sp. strain TAL582SR. Although strain TAL582SR effectively nodulated leucaena seedlings in the sterile peat-vermiculite dibble tube medium, nodulation by this strain was minimal in an unsterile Waialua clay soil. It may be that strain TAL582SR survived poorly in the soil or perhaps was less competitive than the indigenous soil rhizobia.

In the pre-transplanting treatment study, the most important component involved in the improvement of post-transplanting growth was an initial inoculation with G. mosseae. The addition of 10 or 25 ppm starter N alone generated few significant growth advantages. When the two levels of starter N were combined with a G. mosseae inoculation, responses were generally similar to a G. mosseae inoculation without the starter N. Although the effects of the G. mosseae inoculation on plant growth were mostly insignificant at transplanting, within 4 weeks the

inoculated leucaena plants exhibited significantly higher compound leaf numbers and shoot dry weight values. As the leucaena plants achieved similar levels of mycorrhizal infection after transplanting in both inoculated and uninoculated plants, the lead held by the inoculated plants decreased. However, even after 8 weeks of growth in an unsterile Waialua clay soil, the advantages of the initial inoculation with G. mosseae, although diminishing, were still apparent for a number of parameters which included shoot and root dry weight, shoot height, and compound leaf number. It is likely that an unplanned and chronic heat stress in the greenhouse was responsible for the loss of nodulation in this experiment. The G. mosseae infection apparently ameliorated this stress and resulted in minimal leaf losses and chlorosis during the first 4 weeks after transplanting. Similarly, the growth lead imparted by the early G. mosseae infection could be a decisive factor in ensuring the survival of leucaena seedlings in an inclement environment. Although several interesting trends were reported in the experiments which comprised this greenhouse investigation, further work must be conducted in the field to allow a more adequate expression of the full growth potential of L. leucocephala (Lam.) de Wit.

VII. APPENDIX A -- A COMPARISON OF THE EFFECTIVENESS OF WILD AND MUTANT STRAINS OF RHIZOBIUM SP. STRAIN TAL582

An experiment was done to determine if Rhizobium sp. strain TAL582SR retained its ability to infect and fix N_2 as well as its wild parent, TAL582, in association with L. leucocephala (Lam.) de Wit. The experiment was conducted in a growth chamber maintained at a day/night temperature of 26°C/20°C and a humidity of 45%. A 12-hour photoperiod was maintained. Leucaena seeds were separated into two sets; one set was inoculated with TAL582SR and the other with TAL582. After 2 days of preincubation, the peat-covered inoculated seeds were planted in Dispo plastic pouches (Scientific Products) containing a liquid, N-free Azolla medium (Yoshida, 1976). The plants were constantly supplied with this liquid medium over the course of the experiment. Initially, four seeds that were germinating were placed in each pouch. One week after planting, the seedlings were thinned to two per pouch. Sterilized solutions and aseptic techniques were not used here. However, care was taken to prevent cross-contamination of the pouches. Seven replicates were prepared for each treatment. The seedlings were grown for 8 weeks and then harvested. The values for total plant dry weight, nodule dry weight, and nodule number indicate that there were no differences between the wild and mutant TAL582SR strains in terms of infectivity and effectiveness (Table 10). Nodules from plants in both treatments had red interiors and seedlings were green and healthy.

APPENDIX A (CONT.)

Table 10. A comparison of *Rhizobium* sp. strain TAL582 and its streptomycin-resistant derivative, TAL582SR.

	Parameters		
Rhizobium strain	Plant dry weight	Nodule dry Weight	Nodule number
	mg		
TAL582	120.1	3.36	6.92
TAL582SR	135.9	3.95	6.16
C.V.	33.2	42.2	40.6
F values at 0.05 level	NS†	NS	NS

† NS = not significant.

VIII. APPENDIX B -- ASSESSMENT OF INFECTIVITY OF VAM FUNGI

This preliminary experiment was conducted to rate the infectivity of four endomycorrhizal fungi: Glomus etunicatus, Glomus fasciculatus, Glomus mosseae, and Glomus pulvinatus. Spores and sporocarps from the rhizospheres of infected cowpea (Vigna sp.) were obtained by wet seiving and decanting (Gerdemann and Nicolson, 1963; Daniels and Skipper, 1982). Infected root segments (1.0 g) were then mixed into water suspensions of each group of propagules. Dibble tubes containing a sterile peat-vermiculite mixture and L. leucocephala seeds inoculated with Rhizobium sp. strain TAL582 were treated with 15 ml of the mycorrhizal suspensions. The experiment was set up according to the completely randomized design. Seedlings were fertilized twice a week with a 1.5 strength N-free liquid fertilizer (NFLF - Burton et al., 1972) containing 1.5 ppm P. The low P level was chosen to allow adequate development of mycorrhizal infection. The seedlings were given water on the remaining 5 days of the week. The leucaena plants were harvested 2, 3, and 4 weeks after planting. Four replicates were employed to determine infection by the VAM fungi. Root segments were stained (Phillips and Hayman, 1970) and infection was rated qualitatively according to the density of the arbuscules and vesicles present in the roots. Infection was evident in nearly all plants by the second week of growth. Infection ratings from the last sampling are presented in Table 11. Leucaena plants inoculated with G. mosseae showed the highest VAM infection levels.

APPENDIX B (CONT.)

Table 11. Infectivity of VA mycorrhizal species on leucaena.

VAM Fungi	Infection Rating
G. etunicatus	±†
G. fasciculatus	+
G. pulvinatus	++
G. mosseae	+++

† Infection levels: ± = very low, + = low, ++ = fair, +++ = high.

IX. APPENDIX C -- THE INFLUENCE OF PRE-TRANSPLANTING TREATMENTS ON THE EARLY POST-TRANSPLANTING PERFORMANCE OF LEUCAEANA AND ITS N_2-FIXING SYMBIONT, PRELIMINARY STUDY

In this preliminary study, leucaena seeds inoculated with TAL582SR were subjected to a variety of treatments at the time of planting or transplanting. All plants were started at the same time (23 Oct. 1981) in dibble tubes. The 10 treatments employed are discussed in detail below.

1. G. mosseae inoculation -- (M). A dried, high-Ca soil in which corn infected with a pure culture of G. mosseae was grown served as the source of mycorrhizal inoculum. This soil was obtained from the laboratory of Dr. C. Murdock, Horticulture Department. Sprores and sporocarps were obtained by wet seiving and decanting (Gerdemann and Nicolson, 1963; Daniels and Skipper, 1982). The spores and sprorocarps were suspended and diluted in distilled water. Prior to the placement of the leucaena seeds in the dibble tubes, 1 ml of the shaken spore suspension was pipetted onto the sterile peat-vermiculite mixture in the tubes. The number of G. mosseae spores and/or sporocarps per ml of suspension was estimated by dilution to be 25. The seeds were then placed into the dibble tubes and covered with growth medium.

2. Mycorrhizal control -- (Cm). A mycorrhizal control for the above treatment was prepared by forcing the water suspension of G. mosseae through a 5 um Millipore filter. The 5 um filter prevented the passage of fungal spores and sporocarps but allowed the passage of bacteria and other smaller suspended particles. The filtrate was added to the dibble tubes in 1 ml aliquots as in treatment #1.

3. Starter N -- (N). Dibble tube seedlings were drenched with a single application of NH_4NO_3 (4.4 ppm N) in NFLF 1 week after planting. The N applied amounted to approximately 0.14 mg per tube.

4. A second Rhizobium inoculation -- (I). At the time of transplanting, seedlings previously inoculated with Rhizobium sp. strain TAL582SR were reinoculated with TAL582SR in a sterile peat carrier. These bacteria were prepared for the second inoculation by adding 10 ml Vincent's solution to a 1-week-old YMA slant culture and mixing. Several slants were scraped and mixed as described earlier. Each bacterial suspension was added to a sterile Dow ziploc plastic bag containing 50 g of a fine, sterile peat (as used for coating inoculated seeds). Thereafter, an additional 20 ml portion of Vincent's solution was poured into the plastic bags. The peat was thoroughly mixed with the bacterial suspension in each bag and incubated for 3 days at 30°C. After incubation, 2.5 g portions of peat were sprinkled evenly into soil depressions in the 2.6 liter pots before depositing the dibble tube seedlings. To test for Rhizobium viability in the peat, serial dilutions of the inoculated peat were plated out on YMA and an estimated 10^8 Rhizobium per gram of peat were recorded after incubation.

5. Control for second Rhizobium inoculation -- (Ci). The control for the second Rhizobium inoculation was prepared by following the steps outlined above, but eliminating the Rhizobium amendment step.

6. Control -- (C). Leucaena seeds were inoculated with TAL582SR as outlined before. No other treatment amendments were made.

The following treatments consisted of combinations of G. mosseae, starter N, and a second Rhizobium inoculation. The procedures in the

combination treatments were strictly additive so further description of each additional treatment is unnecessary.

7. G. mosseae inoculation plus a second Rhizobium inoculation -- (MI).

8. G. mosseae inoculation plus starter N (4.4 ppm) -- (MN).

9. G. mosseae inoculation plus starter N (4.4 ppm) plus a second Rhizobium inoculation -- (MNI).

10. A second Rhizobium inoculation plus starter N -- (NI).

The contents of the dibble tubes were drenched with 1.5 strength NFLF (Burton et al., 1972) amended or unamended with NH_4NO_3 (4.4 ppm N) 1 week after planting. The P level in the NFLF was reduced from 18 to 1.5 ppm P. Subsequent drenchings were made with the low P NFLF twice a week until transplanting. On the remaining days of the week, the dibble tube seedlings were given water only. After transplanting, 100 ml of the low P NFLF was added to the pots once each week. Watering of the potted plants was as needed.

This experiment was initiated on 23 Oct. 1981. The seedlings were grown for 8 weeks before transplanting into 2.6 liter pots containing an unsterile Waialua clay soil (19 Dec. 1981). Two harvests were made 8 weeks after transplanting (11 and 12 Feb. 1982) and 20 weeks after transplanting (5 and 6 May 1982). Prior to transplanting, seedlings were segregated by treatments in the dibble tube racks but were kept in close proximity to each other. For statistical purposes, these plants were considered completely randomized. After transplanting, the pots were set up according to the randomized complete block design. Pots were spaced approximately 10 cm apart. The winter and spring months were cool compared to the summer months and this was reflected in cooler growing conditions in the greenhouse.

At transplanting and at the two subsequent harvests, 10 plants from each treatment were randomly selected for measurements. From these 10 replicates, shoot and root dry weight, shoot height, nodule number and dry weight, and Kjeldahl total shoot and root N concentrations were measured. Acetylene reduction activity was measured only at 8 and 20 weeks after transplanting. Additional nutrient concentrations in the shoot and roots were measured 20 weeks after transplanting with a vacuum X-ray quantometer. Five replicates were randomly chosen from those mentioned above for these nutrient analyses. Additional plants were collected for determination of mycorrhizal infection (4 replicates). Root samples were collected and stained as in the Materials and Methods section. Unreplicated nodule occupancy tests were performed at 20 weeks after transplanting according to the procedures outlined in the Materials and Methods section. Statistical comparisons were made with the SAS statistical program on the computer (Helwig and Council, 1979).

At transplanting, sparse infection was noted in the seedlings inoculated with G. mosseae and with or without starter N (Table 12). Few arbuscules and vesicles were observed in these root segments. These results may suggest that the number of viable propagules might have been quite low in the dry soil that was used for inoculum. Although a few studies have reported complete VAM infection with very few spores (Daft and Nicolson, 1969; Allen and St. John, 1982), Ferguson and Woodhead (1982) recommend 3 to 5 spores per gram of soil to ensure rapid mycorrhizal colonization. Daniels and Skipper (1982) reported that the drying of soil inoculum reduces spore viability. Also, it is possible that the colonization of the roots took longer because the spores and sporocarps were the sole source of infecting material (Daniels and Skipper, 1982).

Table 12. The influence of pre-transplanting treatments on several growth parameters of leucaena at transplanting.

Treatment†	PARAMETERS							
	Shoot dry weight	Root dry weight	Nodule dry weight/ plant	Nodule number/ plant	Shoot height	Shoot N	Root N	VAM infection
	----------mg----------				cm	-------mg/g-------		%
M	179.50 b§	129.89 ab	8.82 a	10.5 a	7.19 ab	15.94 b	13.85 a	24.5
N	215.90 a	152.97 a	9.09 a	12.6 a	7.75 a	16.96 ab	13.87 a	0
MN	196.91 ab	128.91 ab	8.86 a	14.2 a	7.08 ab	17.06 ab	13.54 a	17.5
C	182.99 b	117.71 b	8.83 a	13.4 a	7.80 a	17.57 a	13.78 a	0
Cm	200.34 ab	118.07 ab	9.70 a	15.6 a	6.81 b	17.76 a	13.69 a	0
C.V.	14.9	26.2	18.6	37.7	10.3	5.6	4.8	-

† M = G. mosseae inoculation, N = nitrogen (4.4 ppm), MN = G. mosseae plus nitrogen (4.4 ppm), C = control, Cm = mycorrhizal control.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

At transplanting, a nonsignificant trend favored shoot and root growth of seedlings given only the starter N (Table 12). No other treatment-related trends were apparent at this time.

Prior to the collection of plant samples at 8 weeks after transplanting, all plants were sprayed with a foliar insecticide, Kelthane 35, to kill spider mites which had invaded a number of the potted leucaena. A few leaves were lost after spraying but sustained visible damage was not incurred.

In the 8 weeks after transplanting, mycorrhizal infection due to indigenous soil VAM fungi tended to equalize the level of infection in all treatments (Table 13). Also, the intensity of infection in the root segments was far greater than at transplanting. Spores were observed in association with root segments in all treatments.

In spite of the equal levels of mycorrhizal infection, the benefit of a G. mosseae inoculation in conjunction with the 4.4 ppm starter N application was observed with regard to shoot and root dry weight, nodule dry weight, and nodule number (Table 13). It is not understood why the similar treatment which employed a G. mosseae inoculation and starter N along with a second Rhizobium inoculation did not give comparable values. It is unlikely that the second inoculation would reduce the values. No significant differences were noted in the shoot height and N concentration parameters (Table 13).

Twenty weeks after transplanting, nodule occupancy by Rhizobium sp. strain TAL582SR was determined. Nodules from a single plant in each treatment were plated out in cluster plates containing YMA, streptomycin sulfate (500 ppm), demosan (50 ppm), and actidione (100 ppm). The presence of this strain was found in only two treatments. These plants,

Table 13. The influence of pre-transplanting treatments on several growth parameters 8 weeks after transplanting.

Treatment†	PARAMETERS							
	Shoot dry weight	Root dry weight	Nodule dry weight/ plant	Nodule number/ plant	Shoot height	Shoot N	Root N	VAM infection
	g		mg		cm	mg/g		%
M	1.01 b§	0.73 b	12.15 b	28.60 b	15.18 a	28.13 a	14.68 a	84.00 a
N	1.04 b	0.80 b	9.30 b	29.90 b	15.15 a	25.67 ab	14.89 a	77.50 a
I	1.05 b	0.83 b	12.88 b	36.70 b	15.12 a	26.78 ab	13.63 a	80.00 a
MI	1.33 ab	0.97 b	13.36 b	35.60 b	15.99 a	25.00 ab	13.79 a	82.50 a
NI	1.13 b	0.88 b	9.26 b	26.20 b	16.12 a	24.86 ab	13.61 a	83.00 a
MN	1.55 a	1.30 a	33.53 a	60.30 a	16.43 a	20.41 b	12.04 a	79.00 a
MNI	1.23 b	0.92 b	13.90 b	27.80 b	16.83 a	25.57 ab	13.76 a	91.00 a
C	1.02 b	0.81 b	13.99 b	33.30 b	15.09 a	27.43 a	14.18 a	79.00 a
Cm	1.06 b	0.87 b	14.41 b	34.40 b	15.79 a	25.48 ab	13.17 a	81.50 a
Ci	1.15 b	0.88 b	12.55 b	31.60 b	16.11 a	26.05 ab	13.52 a	75.50 a
C.V.	29.3	37.0	88.5	66.2	11.9	17.5	14.6	11.3

† M = G. mosseae inoculation, N = nitrogen (4.4 ppm), I = second TAL582SR inoculation, MI = G. mosseae plus second TAL582SR inoculation, NI = nitrogen (4.4 ppm) plus second TAL582SR inoculation, MN = G. mosseae plus nitrogen (4.4 ppm), MNI = G. mosseae plus nitrogen (4.4 ppm) plus second TAL582SR inoculation, C = control, Cm = mycorrhizal control, Ci = control for second TAL582SR inoculation.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

inoculated with G. mosseae or given starter N only, exhibited 2% and 6% infection by TAL582SR, respectively. In YMA plates treated with actidione only (100 ppm), 100% growth was observed. These results further suggest that a second Rhizobium inoculation with TAL582SR does not increase the likelihood of this strain to be maintained as a symbiont in an unsterile Waialua clay soil.

In many parameters for plants harvested 20 weeks after transplanting, no significant treatment differences were recorded and where there were differences, few clear trends could be defined. The lead held by plants inoculated with G. mosseae and given starter N had deteriorated and was nonsignificant at this point in the study (Table 14). At this harvest, unreplicated root samples were stained for mycorrhizal evaluation. Infection was exceptionally dense and was rated between 98% and 100% for the 10 treatments (data not presented). Large numbers of spores were observed in all treatments. Additional nutrient values for the shoot and roots are presented in Tables 15 and 16. Again, treatment differences here were small and often nonsignificant. No trends were evident.

It appears that the major limiting factor at this last harvest made 20 weeks after transplanting was the size of the pots. An examination of the roots showed that most plants in any given treatment were "potbound" and likely restrained from exhibiting normal treatment responses. Linderman and Hendrix (1982) warned that caution is required in the interpretation of results from growth experiments conducted over an extended period, especially where the volume of the growth medium is restricted. Sometimes the plants from one or more treatments may appear to catch up with the growth of plants in another treatment (as was illustrated in this present experiment). Although this could be a

Table 14. The influence of pre-transplanting treatments on several growth parameters 20 weeks after transplanting.

Treatment†	PARAMETERS								
	Shoot dry weight	Root dry weight	Nodule dry weight/ plant	Nodule number/ plant	Shoot height	Shoot N	Root N	C_2H_4/ plant	Specific nodule activity
	g		mg		cm	mg/g		nmoles/hr	nmoles/g hour
M	15.37 a§	15.22 a	427.94 a	601.5 a	64.74 a	13.28 a	9.28 a	552.6 ab	1243.5 a
N	13.46 a	14.65 a	421.80 a	618.0 a	60.97 a	11.34 a	9.22 a	444.2 ab	1044.4 a
I	15.58 a	15.01 a	415.96 ab	572.0 a	65.58 a	12.16 a	9.32 a	508.8 ab	1138.3 a
MI	16.53 a	15.17 a	427.21 a	585.4 a	65.02 a	13.52 a	9.44 a	705.4 ab	1827.3 a
NI	14.73 a	14.71 a	422.69 a	574.6 a	66.17 a	13.68 a	9.72 a	704.8 ab	1598.7 a
MN	15.50 a	14.56 a	446.70 a	627.9 a	60.77 a	12.98 a	8.90 a	854.7 a	1774.2 a
MNI	14.82 a	14.65 a	357.80 ab	498.2 ab	61.92 a	13.70 a	10.02 a	609.2 ab	1631.8 a
C	13.33 a	14.91 a	370.21 ab	529.8 ab	59.32 a	12.34 a	10.12 a	398.3 b	997.4 a
Cm	15.06 a	13.77 a	347.95 ab	488.3 ab	60.46 a	12.52 a	10.16 a	620.7 ab	1274.9 a
Ci	13.13 a	13.05 a	300.01 b	394.4 b	59.11 a	11.68 a	9.98 a	433.3 ab	1250.9 a
C.V.	23.3	14.2	29.9	31.3	18.6	12.9	10.1	70.0	62.6

† M = G. mosseae inoculation, N = nitrogen (4.4 ppm), I = second TAL582SR inoculation, MI = G. mosseae plus second TAL582SR inoculation, NI = nitrogen (4.4 ppm) plus second TAL582SR inoculation, MN = G. mosseae plus nitrogen (4.4 ppm), MNI = G. mosseae plus nitrogen (4.4 ppm) plus second TAL582SR inoculation, C = control, Cm = mycorrhizal control, Ci = control for second TAL582SR inoculation.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

Table 15. The influence of pre-transplanting treatments on the nutrient content of leucaeana shoots 20 weeks after transplanting.

Treatment†	PARAMETERS								
	P	K	Ca	Mg	S	Si	Mn	Fe	Cu
	----------%----------						----------ppm----------		
M	0.13 a§	1.17 a	0.68 a	0.19 a	0.11 a	0.05 b	61.2 b	186.0 ab	15.0 a
N	0.14 a	1.06 ab	0.56 b	0.20 a	0.09 a	0.09 ab	66.8 b	201.0 ab	15.8 a
I	0.14 a	1.03 ab	0.60 ab	0.20 a	0.11 a	0.07 b	66.8 b	244.6 ab	15.4 a
MI	0.13 a	1.14 ab	0.61 ab	0.19 a	0.11 a	0.08 b	65.8 b	190.8 ab	14.8 a
NI	0.14 a	1.11 ab	0.63 ab	0.20 a	0.11 a	0.16 a	93.4 a	306.2 a	12.4 a
MN	0.14 a	1.04 ab	0.57 ab	0.21 a	0.10 a	0.09 ab	70.6 ab	247.0 ab	15.2 a
MNI	0.13 a	1.04 ab	0.57 ab	0.20 a	0.10 a	0.07 b	61.6 b	177.6 ab	16.2 a
C	0.13 a	1.01 b	0.63 ab	0.21 a	0.10 a	0.09 ab	70.8 ab	194.2 ab	14.2 a
Cm	0.13 a	1.09 ab	0.59 ab	0.20 a	0.10 a	0.08 b	61.8 b	218.6 ab	15.2 a
Ci	0.13 a	1.07 ab	0.57 ab	0.21 a	0.09 a	0.11 ab	74.6 ab	148.0 b	16.0 a
C.V.	8.3	9.3	13.4	13.3	16.2	57.0	24.5	47.9	22.9

† M = G. mosseae inoculation, N = nitrogen (4.4 ppm), I = second TAL582SR inoculation, MI = G. mosseae plus second TAL582SR inoculation, NI = nitrogen (4.4 ppm) plus second TAL582SR inoculation, MN = G. mosseae plus nitrogen (4.4 ppm), MNI = G. mosseae plus nitrogen (4.4 ppm) plus second TAL582SR inoculation, C = control, Cm = mycorrhizal control, Ci = control for second TAL582SR inoculation.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

Table 16. The influence of pre-transplanting treatments on the nutrient content of leucaeana roots 20 weeks after transplanting.

Treatment†	PARAMETERS										
	P	K	Ca	Mg	S	Si	Cl	Mn	Fe	Cu	Al
	----------%----------							----------ppm----------			
M	0.11 a§	0.58 a	0.28 a	0.20 ab	0.12 b	0.61 a	0.40 a	183.6 a	2286 a	7.0 a	2548 a
N	0.12 a	0.53 a	0.31 a	0.18 b	0.13 ab	0.42 a	0.37 a	98.8 a	1725 a	8.2 a	2301 ab
I	0.12 a	0.53 a	0.31 a	0.27 ab	0.13 ab	0.72 a	0.36 a	203.4 a	2314 a	6.6 a	2347 ab
MI	0.11 a	0.61 a	0.29 a	0.23 ab	0.13 ab	0.61 a	0.42 a	181.4 a	2126 a	8.8 a	2282 ab
NI	0.12 a	0.56 a	0.30 a	0.26 ab	0.12 b	0.59 a	0.37 a	168.0 a	1935 a	6.6 a	2283 ab
MN	0.12 a	0.56 a	0.30 a	0.22 ab	0.13 ab	0.57 a	0.39 a	142.0 a	2011 a	7.8 a	2195 ab
MNI	0.11 a	0.55 a	0.31 a	0.27 ab	0.12 b	0.57 a	0.39 a	166.4 a	1771 a	7.0 a	2209 ab
C	0.11 a	0.60 a	0.31 a	0.31 a	0.14 a	0.62 a	0.40 a	180.8 a	1792 a	8.0 a	2267 ab
Cm	0.12 a	0.54 a	0.30 a	0.23 ab	0.13 ab	0.61 a	0.38 a	183.2 a	2173 a	7.0 a	2267 ab
Ci	0.12 a	0.53 a	0.29 a	0.20 ab	0.13 ab	0.42 a	0.39 a	119.6 a	1577 a	8.0 a	2056 b
C.V.	8.7	11.7	8.2	30.3	9.5	35.4	12.1	47.4	29.1	24.1	13.6

† M = *G. mosseae* inoculation, N = nitrogen (4.4 ppm), I = second TAL582SR inoculation, MI = *G. mosseae* plus second TAL582SR inoculation, NI = nitrogen (4.4 ppm) plus second TAL582SR inoculation, MN = *G. mosseae* plus nitrogen (4.4 ppm), MNI = *G. mosseae* plus nitrogen (4.4 ppm) plus second TAL582SR inoculation, C = control, Cm = mycorrhizal control, Ci = control for second TAL582SR inoculation.

§ Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

legitimate result, especially in the field, Linderman and Hendrix (1982) noted that for container experiments it could merely be caused by the faster-growing plants becoming "potbound" or by nutrient depletion.

A very important antagonism which may have had some impact on treatment responses became apparent by the final harvest. Virtually every plant sampled had at least rudimentary signs of root knot nematode infection. At the previous sampling, the plants may have already been attacked by these parasites but there were few, if any, signs to denote their presence in the plants. Egg-laying females were identified as Meloidogyne sp. About 25% of the plants exhibited severe infestation with blackened areas and gall tissue formation. Those plants that were badly infested generally performed more poorly than did those that showed no or only incipient invasion by nematodes. To determine if the nematodes compromised treatment responses, badly infested plants were removed from the pool of data and new analyses of variances and Duncan's multiple range tests were run. Removing these plants did not modify treatment differences significantly so the data is not presented. In several studies, root pathogens have been shown to mask effects due to VAM fungi in growth response experiments (Roncadori and Hussey, 1977; Linderman and Hendrix, 1982). Root knot nematodes have been specifically implicated in yield depressions in a number of legume species (McGinnity et al., 1980). In Hawaii, the presence of parasitic root knot nematodes in field-grown leucaena is not at all uncommon (A. Martinez, personal communication).

Although a few meaningful treatment responses were recorded in this preliminary study, many of the results presented here do not allow adequate conclusions to be made with regard to the beneficial effects of pre-transplanting treatments. A lack of response to the second Rhizobium

inoculation with TAL582SR suggests that this remedial step is unnecessary and does not increase the percentage of nodules that are occupied by this strain. The use of another more persistant Rhizobium strain might have been suggested for follow-up work, but when these experiments were conducted, I was not aware of any superior Rhizobium strains that could be substituted for the strain used. Future experiments must be harvested before the leucaena plants become "potbound". The interaction between the G. mosseae inoculation and starter N appliciation that was shown at 8 weeks after transplanting advocates further experimentation with these treatments. Also, a larger number of fresher VAM propagules should be used to achieve a higher level of infection prior to transplanting. Further examination of the minimal levels of starter N needed to initiate a growth response was considered necessary before testing the effects of starter N alone and in conjunction with a G. mosseae inoculation (see Appendix D).

X. APPENDIX D -- THE EFFECTS OF A SINGLE APPLICATION OF INORGANIC N ON DIBBLE TUBE-GROWN LEUCAENA

The experiment recorded in Appendix C indicated that before additional work pertaining to interactions between mycorrhizal infection and starter N was initiated, more had to be learned about the effects of a single application of starter N on the growth of the dibble tube seedlings. This short experiment was conducted to determine the effects of eight levels of starter N on the leucaena seedlings and the Rhizobium symbiont. Leucaena seeds were inoculated with TAL582SR and planted in dibble tubes containing a sterile peat-vermiculite medium. One week after planting, the seedlings were drenched with 1.5 strength N-free liquid fertilizer (NFLF - Burton et al., 1972) containing 0, 4.4, 10, 25, 50, 100, 200, and 500 ppm N. The source of N was NH_4NO_3. The seedlings were thereafter fertilized twice a week with NFLF. Plants were supplied with water for the remaining 5 days of the week. The plants were completely randomized on racks and were harvested after 6 weeks of growth. An analysis of variance and a Duncan's multiple range test to compare treatment means were made using the SAS statistical package on the computer (Helwig and Council, 1979). Mean comparisons for plant dry weight, nodule dry weight and number, Kjeldahl N and total N uptake were based on 10 replicates per treatment and are presented in Table 17.

The two highest additions of starter N, 200 and 500 ppm N, yielded significantly greater amounts of dry plant material than smaller applications. Over the range of treatments, the nodulation parameters did not respond significantly. Plant N concentrations in the various treatments did not express any differences and the N uptake reflected the plant dry

APPENDIX D (CONT.)

Table 17. The effects of a single application of inorganic N at planting on several growth parameters of dibble tube-grown leucaena.

N level	Plant dry weight	Nodule dry weight/ plant	Nodule number/ plant	Plant N	N uptake
ppm	----------mg----------			mg/g	mg/plant
0	157.6 cd†	7.89 abc	12.1 a	9.42 a	1.40 c
4.4	166.9 cd	8.02 abc	11.8 a	11.39 a	1.82 bc
10	153.6 d	6.55 bc	15.2 a	9.28 a	1.60 bc
25	165.3 cd	5.63 c	11.4 a	10.45 a	1.71 bc
50	181.7 cd	8.90 ab	13.0 a	10.99 a	2.09 abc
100	189.8 c	8.21 ab	14.2 a	9.94 a	2.09 abc
200	220.6 b	10.31 a	16.9 a	10.78 a	2.34 ab
500	259.6 a	8.24 ab	11.0 a	9.61 a	2.73 a
C.V.	17.8	32.2	43.4	12.5	20.7

† Means within columns followed by the same letter do not differ significantly at the 5% level of probability according to the Duncan's multiple range test.

weight parameter. Based on these results, larger seedlings could be generated by applying an appropriate amount of starter N. However, the starter N did not affect nodulation in either a positive or negative manner. Although a range of 4.4 to 500 ppm N was applied to the dibble tubes, perhaps not all of the N was available to the seedlings. Some N may have been leached out of the medium with subsequent waterings. Since I wanted to corroborate results obtained with 4.4 ppm N in Appendix C, I decided to use slightly higher (but not significantly greater) levels of starter N, i.e., levels that did not cause differences in plant dry weight production.

Table. 18. The effects of age and method of propagation on several growth paramters of leucaena at the time of transplanting.

Age (days)†	Method of propagation§	PARAMETERS									
		Shoot dry weight	Root dry weight	Shoot/root ratio¶	Nodule dry weight/ plant	Nodule number/ plant	Shoot height	Shoot N	Root N	--- N uptake ------ shoot	root --
		---------- g ---------			mg		cm	------ mg/g ------		----- mg/plant ----	
25	DS	0.08	0.02	4.20	0.75	6.83	8.08	29.22	18.95	2.34	0.40
25	T	0.06	0.02	3.93	1.08	8.67	9.20	25.18	17.63	1.51	0.27
35	DS	0.22	0.09	2.67	1.32	10.67	10.21	25.54	16.87	6.18	1.44
35	T	0.11	0.05	2.43	3.02	7.33	6.67	16.20	12.69	1.81	0.61
62	DS	0.85	0.58	1.55	22.10	52.33	16.02	25.77	13.05	21.89	7.57
62	T	0.20	0.15	1.36	5.55	8.33	8.18	21.37	14.24	4.28	2.13
90	DS	1.54	1.91	0.92	117.18	405.00	17.37	17.63	9.88	26.98	18.86
90	T	0.15	0.21	0.73	6.27	10.83	9.27	19.98	11.82	3.00	2.43
C.V.		54.3	88.8	29.1	67.1	79.6	27.3	6.3	12.1	20.9	12.1
$LSD_{0.05}$		0.25	0.39	0.76	15.38	59.19	3.38	3.29	4.01	4.09	1.17

† Age of plants started from direct-seeding or age of seedlings to be transplanted.
§ DS = direct-seeded, T = transplanted.
¶ The shoot/root ratios are not exactly equal to shoot mean/root mean in the table due to rounding errors and difference in variances of the shoot and root components of the ratio.

Table. 19. The effects of age and method of propagation on several growth parameters of leucaena at the time of harvest.

Age (days)†	Method of propagation§	PARAMETERS: Shoot dry weight	Root dry weight	Shoot/root ratio¶	Nodule dry weight/ plant	Nodule number/ plant	Shoot height	Shoot N	Root N	--- N uptake -- shoot	root	C_2H_4/ plant	Specific nodule activity
		-------- g --------			mg		cm	----- mg/g -----		--- mg/plant ---		nmoles/ hour	nmoles/ g·hour
25	DS	3.98	3.42	1.29	133.9	394.0	28.61	15.36	9.87	69.39	38.27	426.5	3821.7
25	T	3.39	2.49	1.41	70.4	154.7	30.90	20.02	11.20	70.14	28.91	536.8	6826.2
35	DS	3.61	3.54	1.05	203.3	632.2	24.84	13.41	8.67	49.76	32.33	466.2	2366.0
35	T	3.44	2.62	1.34	83.6	169.9	31.17	19.31	10.93	73.52	31.62	572.7	7102.3
62	DS	4.49	4.71	1.17	300.4	929.1	26.28	11.73	7.88	52.74	35.54	584.1	2023.4
62	T	4.99	3.61	1.45	119.7	331.7	40.19	19.87	11.88	91.79	40.41	873.1	7187.4
90	DS	4.78	5.98	0.82	416.7	1425.8	33.31	12.85	7.62	62.82	49.37	657.2	1617.4
90	T	5.31	4.29	1.24	149.9	355.7	42.41	19.62	10.60	95.79	43.96	1042.1	6891.7
C.V. (a)#		26.2	26.8	27.9	30.6	33.3	17.8	14.3	7.7	20.2	22.0	36.8	31.1
C.V. (b)††		21.9	30.1	24.0	35.0	35.8	15.4	8.5	8.0	22.6	27.0	43.4	26.3
$LSD_{0.05}$(A)§§		0.84	1.04	0.28	58.19	176.77	4.50	1.89	1.05	21.43	13.56	254.50	1131.79
$LSD_{0.05}$(A X MP)¶¶		0.94	0.99	0.26	55.19	212.72	4.83	2.65	1.04	19.87	12.51	237.14	1243.77

† Age of plants started from direct-seeding or age of seedlings to be transplanted.
§ DS = direct-seeded, T = transplanted.
¶ The shoot/root ratios are not exactly equal to shoot mean/root mean due to rounding errors and differences in variances of the shoot and root components of the ratio.
# Degree of precision attached to the measurement of the mainplot treatment (age) effects.
†† Degree of precision attached to the measurement of the subplot treatment (method of propagation) and interaction effects.
§§ Used for comparisons between methods of propagation at the same age.
¶¶ Used for comparisons of age within method of propagation.

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